

Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.

95-
522452

RECEIVED

STA/STA

REPORT OF THE
NORTHERN REGIONAL RESEARCH CENTER

May 1981

JAN 28 '83

PROGRESSIVE PUBLICATIONS
CURRENT SERIAL RECORDS

North Central Region
Agricultural Research
Science and Education Administration
UNITED STATES DEPARTMENT OF AGRICULTURE

Mention of trade names or company names is solely to provide specific information and does not constitute guarantee or warranty by the U.S. Department of Agriculture or an endorsement by the Department over others not mentioned.

When reporting research involving pesticides, this publication does not imply that pesticide uses discussed here have been registered. Registration is necessary before recommendation. Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.



CONTENTS

	<u>Page</u>
I. INTRODUCTION.....	1
II. SELECTED ACCOMPLISHMENTS.....	4
III. BIOMATERIALS CONVERSION LABORATORY.....	12
A. Technologies for Food and Feed Uses of Field Crops.....	12
1. Basic engineering studies on preparation of soy foods....	12
2. Fundamental studies on separation of starch, protein, and lipid of corn.....	14
3. Development of mycostatic systems to permit safe, low- energy grain drying.....	16
B. Technologies for Industrial Uses of Plant and Animal Products.....	17
1. Starch-based copolymers for making elastomers.....	17
2. Starch-based purification aids for wastewater.....	17
3. Flame-resistant polyurethane foams and biodegradable films and packaging prepared from starch.....	18
4. Controlled release of pesticides by formulating with cereal-based starch and flour derivatives.....	20
5. Graft polymers of starch for agricultural chemicals and absorbents.....	21
6. Plant component separation and physical characterization.....	24
7. Basic studies on modification of natural polymers as replacements for petroleum-derived polymers.....	25
8. Basic studies on biopolymers for improving safety of pesticides.....	25
9. Hydrocarbon-producing plants as potential multi-use crops.....	27
10. Increased energy efficiency of substrate preparation for alcohol fermentations.....	27
11. Innovative fermentation technology for alcohol production.....	27
C. Technologies and Products to Increase Exports of Agricultural Products.....	27
1. Principles underlying design of foods blends for the export market.....	27

	<u>Page</u>
IV. CEREAL SCIENCE AND FOODS LABORATORY.....	30
A. Technologies for Food and Feed Uses of Field Crops.....	30
1. Interactions of food carbohydrates.....	30
2. Corn starches--physical characteristics and biological digestibilities.....	31
3. Fundamental studies on separation of starch, protein, and lipid of corn.....	32
4. Interactions of dietary fibers from cereal products with mutagens in digested food.....	32
5. Methods of analysis to facilitate genetic improvements of cereal grain protein.....	33
6. Epoxides from lipid hydroperoxides and their interactions in cereal and oilseed foods.....	36
7. Molecular structure of Maillard-type browning reaction products.....	37
B. Technologies for Industrial Uses of Plant and Animal Products.....	40
1. Enzymatic conversion of cellulose to sugars for alcohol fermentations.....	40
2. Energy-saving methods for recovery of usable protein from alcohol or methane fermentation media.....	41
C. Chemical Residues and Additives in Food and Feed.....	41
1. Effect of environmental contaminants on cereal foods and feeds.....	41
D. Food Composition and Improvement.....	42
1. Action of human digestive system upon cereal grain fiber sources and related foods.....	42
V. FERMENTATION LABORATORY.....	44
A. Physiological and Biochemical Technology To Improve Crop Production.....	44
1. Polysaccharides in specific associations of nitrogen-fixing microbes with plants.....	44
2. Physiology of nitrogen-fixing blue-green algal and rhizobial symbioses with plants.....	47
3. Nitrogen contribution of <u>Azolla</u> ssp. in aquatic farming systems.....	49

	<u>Page</u>
B. Biological Agents for Pest Control.....	49
1. Insecticidal preparations of <u>Bacillus thuringiensis</u> and other microbial insect pathogens.....	49
C. Technologies for Food and Feed Uses of Field Crops.....	51
1. Rapid characterization of yeasts through genetic and DNA/DNA hybridization and computer analysis.....	51
2. Germ plasm bank of microorganisms for research on plant residue utilization.....	53
3. Effect of immobilization procedure and carrier on enzymes that hydrolyze cereal food polymers.....	55
4. Characterization and classification of Mucorales from cereal grains and their raw products.....	57
5. Fermentative utilization of cane sugar baggasses.....	59
6. Thermophilic microbial conversion of cellulosic materials to animal feed.....	60
D. Technologies for Food and Feed Uses for Animal Products.....	60
1. Conversion of feedlot wastes into feed supplements by fermentation with grain.....	60
E. Technologies for Industrial Uses for Plant and Animal Products.....	62
1. Increased energy efficiency of substrate preparation for alcohol fermentations.....	62
2. Innovative fermentation technology for alcohol production.....	62
F. Technologies and Products to Increase Exports of Agricultural Products.....	62
1. Soybean foods of the traditional Oriental type for the export market.....	62
G. Natural Toxicants and Microbial Toxins.....	64
1. Germ plasm bank of microorganisms for research on microbial toxins.....	64
2. Aflatoxin and other mycotoxins in corn and other cereal grains.....	66
3. Metabolites of toxin-producing fungi found in corn and other cereal grains.....	71
4. Origin and ecology of mycotoxin-producing fungi in grain.....	75

5. Microbial species interactions and development of aflatoxin in preharvest corn.....	76
6. Establishment and identification of toxic metabolites of certain molds and aflatoxin in livestock fodder.....	77
VI. HORTICULTURAL AND SPECIAL CROPS LABORATORY.....	79
A. Breeding and Production of Forage Crops for Hay, Pastures, and Other Uses Including Turf.....	79
1. Chemicals in tall fescue affecting livestock health and forage utilization.....	79
2. Bioassay of chemical constituents of tall fescue forage..	79
B. Introduction, Classification, Maintenance, Evaluation, and Documentation of Plant Germplasm.....	80
1. Chemical analysis of uncultivated plants.....	80
2. Biologically active plant constituents for pest control and medicine.....	84
3. Major fatty acids from Indian seed oils and their possible industrial use.....	86
C. Physiological and Biochemical Technology to Improve Crop Production.....	87
1. Plant cell and tissue culture for the bioproduction of valuable chemicals.....	87
2. Photosynthetic pigments and primary photoacts for increasing efficiency and yield of crops.....	89
D. Technologies for Food and Feed Uses for Field Crops.....	93
1. Composition and properties of seed lipids for foods and feeds.....	93
2. Soybean analysis for improved quality.....	95
E. Reclamation and Revegetation of Land Areas Disturbed by Man..	96
1. Trace element uptake and distribution in agricultural crops grown on disturbed lands.....	96
F. Utilize, Manage, and Conserve Soil Fertility for Increased Production and Nutritional Quality of Plants and Animals.....	98
1. Improve and implement the determination of isotopic nitrogen in soil samples.....	98

	<u>Page</u>
G. Natural Toxicants and Microbial Toxins.....	98
1. Cattle feeding tests of crambe meal as a protein concentrate.....	98
2. Inheritance of glucosinolates in crucifer vegetables....	98
3. Natural toxicants in horticultural crops and cruciferous feeds.....	99
VII. OILSEED CROPS LABORATORY.....	102
A. Technologies for Food and Feed Uses for Field Crops.....	102
1. Effects of vegetable and animal trypsin inhibitors in long-term animal feeding studies.....	102
2. Improving food quality of soy oil products and their stability to heat- and light-catalyzed oxidation.....	103
3. Nutritional quality, safety, and flavor aspects of soybean protein products.....	107
4. Analytical and structure studies of soybean proteins....	111
5. Nutritional and physiological studies of soybean hemagglutinins.....	113
B. Technologies for Industrial Uses for Plant and Animal Products.....	114
1. Chemical modification of soybean oil and its derivatives.....	114
C. Technologies and Products to Increase Exports of Agricultural Products.....	117
1. High-temperature soybean cooking oils for the export market.....	117
2. Soybean oil quality for export markets.....	120
3. Heavy metals in soybeans grown on sewage sludge-amended soil.....	126
D. Human Requirements for Nutrients.....	126
1. Biochemical behavior of isomeric fats in hydrogenated soybean oil.....	126

VIII. NORTHERN AGRICULTURAL ENERGY CENTER.....	135
A. Technologies for Industrial Uses for Plant and Animal Products.....	135
1. Hydrocarbon-producing plants as potential multi-use crops.....	135
2. Increased energy efficiency of substrate preparation for alcohol fermentations.....	137
3. Innovative fermentation technology for alcohol production.....	142
4. Energy-saving methods for recovery of usable protein from alcohol or methane fermentation media.....	145

PROGRESS REPORT
OF THE
NORTHERN REGIONAL RESEARCH CENTER
MAY 1981

INTRODUCTION

The Northern Regional Research Center, located at Peoria, Illinois, is one of the major research centers of the North Central Region, Agricultural Research, Science and Education Administration (SEA-AR), U.S. Department of Agriculture. Basic and applied research is conducted in the physical and biological sciences and in engineering. Northern Center scientists cooperate with representatives of colleges and universities, State experiment stations, research institutes and associations, industrial organizations, and with other Government agencies. Much of the cooperation is informal, but some work is conducted under cooperative agreements and memorandums of understanding. In addition, the Center's program is supplemented by research projects in foreign countries under Public Law 480 grants.

Providing scientific information for improvements in the post-harvest sector of American agriculture is a major mission of NRRC. Such improvements in the handling, storing, processing, and distribution technology through which agricultural products move from production to ultimate use inevitably benefit both consumers and farmers. For example, research on this complex technology provides the basis for preserving and increasing food quantity, economy, quality, safety, and nutritive value. This research also offers our best hope for substantially reducing the energy intensiveness of post-harvest operations, which collectively require at least twice as much energy as is consumed on farms, and for innovations contributing to maintenance of environmental quality. Responding to these needs and opportunities, NRRC's research program and scientific staff provide SEA-AR's principal effort and expertise in processing and utilizing soybeans, cereal feed grains, and special crops. In addition, the Center is devoting attention to alternative energy resources from agriculture through its involvement in the Northern Agricultural Energy Center.

Currently most of the soybean research is directed toward decreasing the cost and improving consumer acceptability and nutritional quality of the oil and of high-protein products derived from the meal. This research continues to justify the stature earned by past accomplishments which provided much of the fundamental basis for today's edible soybean oil and food-grade soy protein industries. For instance, Center scientists pioneered in development of statistically reliable taste-panel procedures that are essential for attaining improved flavor of soy-based foods. In the non-food area, a nucleus still exists of a former sizable effort to exploit soybean and other vegetable oils as petrochemical-sparing industrial raw materials. From this nucleus the research could be expanded rapidly, should national priorities so mandate.

Cereal grain research, on the other hand, is almost equally concerned with industrial and with feed and food uses. For example, Center scientists' special knowledge of the complex chemical and physical properties of corn starch and flour has enabled them to develop new technologies with promising on-farm applications. These include starch-containing plastic film for biodegradable mulches and controlled release of pesticides through encapsulation with derivatized starch. In the food area studies are underway on food fiber aimed at improving the healthfulness of the American diet. In addition, the Center staff continues to be a principal source of information and research on blended foods, such as CSM (from corn, soybean flour, and dry milk solids) for the Food for Peace program. Another major contribution to food science is research to determine the relationship between wheat proteins and their functional properties in bread and other baked goods. Ultimately these studies will provide fundamental insight relevant to nutritional products from other cereals as well as from oilseeds.

A key research resource, the Agricultural Research Culture Collection (NRRL), is a world renowned repository of agriculturally and industrially important microorganisms. Reference cultures, cataloged taxonomic data, and professional expertise associated with this microbial germ-plasm bank have enabled NRRC to make vital contributions and to assume preeminent roles in mycotoxin research and in fermentation technology, including production of food ingredients and fermented foods such as tempeh. Because of the unique capabilities of a multi-disciplinary staff and the importance of the problem, research on mycotoxins has become one of the largest components of the Center's overall effort. This research plays a key role in protecting our food supply from these hazardous substances.

Post-harvest processing behavior and product quality are markedly affected by preharvest factors. Recognizing this, Center chemists, engineers, microbiologists, and physicists participate in joint projects with other SEA-AR and SAES scientists conducting genetic and agronomic studies. Determination of processing and compositional characteristics of plant materials from botanical collections, breeding programs, and studies of soil and atmospheric variables is a major form of such participation. Another involves natural toxicants. Center scientists provide analytical and biochemical information necessary to make sure levels of these minor constituents are not seriously increased in new varieties. This work, like the mycotoxin research, helps assure the safety and nutritional quality of our food supply.

Center scientists have added their weight to the growing emphasis on photosynthesis, nitrogen fixation, and plant tissue culture. Their novel biochemical, microbiological, and physical approaches complement longer standing studies by plant physiologists and thereby expand and diversify the total SEA-AR attack in these high priority areas.

To take advantage of the resources and expertise available at NRRC, one of the two new SEA energy centers, the Northern Agricultural Energy Center (NAEC), is headquartered here. The goal of NAEC is to discover, develop,

and demonstrate technology which will permit agriculture to be energy self-sufficient on a net basis by 1990 under conditions that sustain productivity. The mission of the portion of the Energy Center program being carried out at NRRC is: (1) to develop innovative fermentative techniques emphasizing new microorganisms, new fermentation configurations, and use of substrates such as distressed grains; (2) to examine new approaches to substrate preparation and particularly investigate chemical, physical, and microbiological methods for conversion of cellulosic plant residues to sugars and thence to alcohol; (3) to evaluate the food and feed potential of nutrients isolated from fermentation residues and to develop methods to recover protein from these residues; and (4) to expand the evaluation of plants as sources of hydrocarbons and to develop methods to separate and isolate hydrocarbon plant constituents.

This report summarizes research of the Center during calendar year 1980 and lists publications and patents resulting from the research. The research summaries include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are available to those having a special interest in the development of public agricultural research programs.

This report was prepared at the Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604. Additional copies of the report and reprints of most publications can be obtained from the Northern Regional Research Center. A separate annual listing of publications and patents also is available.

SELECTED ACCOMPLISHMENTS

Drying High-Moisture Corn. The successful development of the "Trickle Ammonia Process" for preserving and ambient air drying of high-moisture corn, using small amounts of ammonia to control mold growth, led to research on alternative mycostatic agents. Sulfur dioxide was found to be effective in controlling mold propagation on high-moisture corn. EPA clearance to permit use of gaseous sulfur dioxide as a fungicidal agent was granted in 1980, following their approval of ammonia for the same purpose in 1979. American farmers are now provided with another means for preserving and drying high-moisture corn. [See Biomaterials Conversion Laboratory (BC), A.3.]

Plastics from Plants. Basic research at NRRC on the concept of using renewable natural polymers to replace petroleum-derived materials has resulted in new, biodegradable plastics. The potential is excellent for applying the technology to development of biodegradable mulch, planters, and containers urgently needed by farmers. The new material is made from the natural polymer, starch, by expanding its molecules with water, heat, and ammonia and then suddenly reducing the temperature in the presence of other compatible polymers. The result is a composite of natural and synthetic material that is strong, flexible, and biodegradable. About 30 million pounds of plastics now are produced annually; replacement of some of these materials with plant-derived plastics can decrease our dependence on finite petroleum and alleviate the negative impact on the environment caused by discarded film and containers made from petrochemicals. (See BC, B.3.)

Encapsulated Pesticides Promising in Field Tests. Expanded field testing demonstrated that encapsulating pesticides with natural polymers is a practical method of improving pest control. These studies, conducted by NRRC in cooperation with scientists at Purdue and other government locations, provided a basis for further modifications of the natural polymer system. Thiocarbamates and DBCP performed best when completely encapsulated. Trifluralin was more effective when half of the herbicide was not encapsulated and thus was available for immediate action. Preliminary data suggest that the new formulations control weeds with less herbicide and without soil incorporation. (See BC, B.4)

Method for Determining Protein Quality. Methodology was developed for estimating protein quality of corn-soy-milk (CSM) by using a colorimeter. Hunter "L" values (light-to-dark) provided a rapid procedure for predicting protein efficiency ratio (PER). Correlation with PER's obtained from rat feeding tests and Hunter "L" values was 0.95. The method has proved to be quite reliable and saves both time and costs of PER assays. (See BC, C.1.)

New Techniques for Measuring Amylose Content of Starch Granules. This research has developed a different technique for measuring the binding of iodine by amylose and starch. The amount of iodine bound by amylose is increased by 50% over conventional iodine titrations and is 15-20% greater than the expected theoretical amount. Of practical interest, the increased

binding improves sensitivity and has allowed us to more accurately determine the amylose content of different distributions of starch granules. [See Cereal Science and Foods Laboratory (CSF), A.2.]

Genetic and Functional Differences of Cereal Proteins Related to Structures. Alcohol-soluble proteins called prolamines are found in all cereal grains, in which they serve as reserve nutrients for the germinating seed and contribute to nutritional and functional characteristics of the grain flours. The properties of these protein chains depend not only on the amounts of the different amino acids within them but also on the order in which the amino acids are linked together. The assembling of the amino acids to form different proteins is controlled by separate genes. We have determined the partial sequence in which the amino acids are arranged in prolamins separated from different grains. Our findings indicate that the closely related grains of wheat, barley, and rye have some prolamins with similar sequences. Likewise, corn, gama grass, and sorghum have similar sequences in their prolamins. Unique sequences explain functional differences between proteins of different cereal grains and may be responsible for differences in dough performance among wheat varieties in bread making. These studies indicate the extent to which evolutionary and genetic changes result in modification of cereal proteins. (See CSF, A.5.)

Dietary Fiber Influences Mineral Requirement of Humans. Cooperative studies with SEA-AR Human Nutrition Research Laboratory, Grand Forks, ND, indicate that feeding human volunteers about 26 g per day of various fiber sources does affect trace mineral requirements. A pooling of all data thus far obtained indicates that: ingestion of fiber sources (i.e., AACC soft white wheat bran, Waldron hard red spring wheat bran, dry milled corn bran, soy hulls, apple powder, carrot powder, or textured soy protein) significantly increased apparent requirements for calcium, phosphorus, iron, and copper. A diet higher in protein (i.e., 15% of energy from protein compared to 8% energy from protein) increased requirements for calcium, phosphorus, zinc, and copper. Thus, dietary fiber content of the diet would be expected to be important in human mineral requirements when available diets are marginal in required trace minerals. (See CSF, D.1.)

Toxicity of Spore-Free Mutants of *Bacillus thuringiensis*. Crystals produced by Spo- Cr⁺ mutants of *B. thuringiensis* were shown to be toxic toward larvae of the European corn borer. The solubilized protein from the crystal is also toxic toward cultured insect cells from the spruce budworm. Mutant toxicity in either case was equal to the toxicity of the parent strain (from which the mutants were derived). These results show that the entomocidal character is conserved in Spo- Cr⁺ mutants of *B. thuringiensis*, and suggests their use as a biological insecticide which avoids the spread of a living agent (the spore). [See Fermentation Laboratory (F), B.1.]

Characteristics and Species Concepts in the Mucorales. In the past, some workers have used sporangiospore size to distinguish certain species of *Rhizopus*. Finding that the characteristic can be significantly varied in *Rhizopus delemar* by different media and temperatures of incubation has helped to explain apparent discrepancies in those workers' reports.

Collecting and studying new strains of Rhizopus and related genera of Mucorales from worldwide locations and various substrate sources helps lay the groundwork for clarifying natural morphological and biochemical variation within genera and species. The studies pinpoint those strains not readily fitting current species concepts so that their detailed examination can lead to modifying our species concepts to greater usefulness by readily including or distinguishing a greater number of naturally occurring strains. (See F, C.4.)

Pathogen Removal by Fermentation. Mycobacterium paratuberculosis was killed within 10 hours in the continuous fermentation of corn with feedlot waste liquid; none were recovered in the product. This demonstrates that pathogens which cause diseases like Johne's disease or tuberculosis are killed in the fermentation and present no problem in refeeding animal waste using this process. (See F, D.1.)

Agricultural Research Culture Collection (NRRL) Now Designated as International Depositary Authority. On January 31, 1981, the NRRL Culture Collection officially acquired the status of International Depositary Authority as established by the World Intellectual Property Organization. The designation recognizes the Collection as one of about one dozen throughout the world suitable for deposition of strains of microorganisms involved in international patents and attests to the scientific and administrative competency of its staff over the years. The NRRL Collection has been active since 1948 as one of only two U.S. collections recognized by the U.S. Patent and Trademark Office as suitable for the deposition of U.S. patent strains. Research and service activities of the staff over that time have contributed to development of policies and proper operational procedures for handling these important microbial germplasm resources. (See F, G.1.)

Measurement of Airborne Aflatoxins During Handling of Contaminated Corn. In 1979, dust and corn samples were collected from an elevator and three farms at harvest in the Southeast. The average aflatoxin level in settled dust samples collected at seven locations in the elevator was 222 p.p.b. The average aflatoxin level in airborne dust samples collected as trucks were delivering corn to and unloading corn from the elevator was 173 p.p.b. The average level in the bulk corn being delivered and unloaded was 78 p.p.b. Aflatoxin levels in harvested corn from the three farms were 18, 37, and 406 p.p.b.; aflatoxin levels in airborne dust samples collected at harvest on same three farms were 16, 32, and 114 p.p.b. On the three farms, there was very little dust in the air-conditioned cabs of the combines; so farmers had little exposure to dusts during harvest. Dust particles from aflatoxin-contaminated corn were coarse and aflatoxin levels were highest in the larger particles. Larger particles would not be absorbed in the lungs and respiratory protection from large particles would be easier than from small particles. Results of these studies indicate that farmers and elevator workers should take appropriate precautions when handling corn known to contain high aflatoxin levels. (See F, G.2.)

Analysis of Aflatoxin-Contaminated Animal Tissues. Animal tissues from a steer fed aflatoxin-contaminated feed were assayed. Aflatoxin B₁ and M₁ were the predominant toxins found in liver, kidneys, heart, spleen, and skeletal muscle (flank). In all tissues except kidneys, B₁ was present in higher levels than M₁. The level of M₁ was 3.5 times the level of B₁ in the kidneys, which suggests that this organ may be the site of B₁ transformation to M₁. The presence of aflatoxins in the edible tissues of bovine presents a possible human health problem that needs further study. (See F, G.2.)

Isolation and Purification of Deoxynivalenol and a New Trichothecene. Deoxynivalenol and a new trichothecene were isolated from corn inoculated in the field with Fusarium graminearum. Compounds were isolated from crude corn extracts by preparative HPLC and purified by reverse phase HPLC. The new trichothecene was characterized by GC and MS of the compound and its trimethylsilyl derivative, which indicate it to be a dihydroxy-12,13-epoxytrichotec-9-ene-8-one. The isolation and purification of deoxynivalenol has provided a pure reference standard of this scarce mycotoxin. The characterization of the new trichothecene has indicated possible interferences with deoxynivalenol analysis and indicated means of avoiding them. (See F, G.2.)

Xanthomegnin Production and Isolation Techniques Developed. Conditions that lead to efficient production of xanthomegnin, a metabolite of Penicillium viridicatum implicated as a possible nephrotoxic agent in swine, have been established. The mold is grown on rice at 10°-15°C for 22 days to produce up to 2 g/kg of rice. Partially refined culture extracts are subjected to a single chromatographic purification utilizing preparative high performance liquid chromatography. This technique rapidly affords multigram quantities of crystalline xanthomegnin that are available for chronic toxicological evaluations not only in rodents but in swine as well. (See F, G.3.)

Aspergillus flavus from Ancient Rodent Seed Cache Economy Does Not Produce Sclerotia. Intensification of an ecological approach to microbiological research has resulted in the interesting finding that A. flavus strains isolated from an ancient rodent seed cache economy do not produce sclerotia, whereas those from native and agricultural ecosystems produce these survival stages. The findings suggest that the cache strains have become rodent-domesticated and morphologically distinct. In another study, aflatoxins and cyclopiazonic acid were detected in sclerotia for the first time. (See F, G.4.)

Quantitative HPLC of Carbonyl Containing Lipids. The separation and quantitation of fatty acids, monoglycerides, diglycerides, and triglycerides have been accomplished by high performance liquid chromatography (HPLC) using gradient elution and infrared detection of the carbonyl functionality. This technique replaces older, more time-consuming methods and provides a means of quantitating other carbonyl-containing entities in seed oils directly without prior isolation or derivatization. This development has applications beyond the analysis of seed oils in such areas as natural products, emulsifier additives, and food chemistry. [See Horticultural and Special Crops Laboratory (HSC), B.1.]

Taxanes from Taxus wallichiana. A total of six new cytotoxic substances of the taxane series have been isolated from T. wallichiana and characterized. These compounds are structurally related to taxol, a leading contender among compounds under consideration for cancer chemotherapy. However, they represent different functionalization of the taxane system with a range of polarities that may extend the chemotherapeutic usefulness of this class of compounds. (See HSC, C.2.)

Divalent Cation Effects on Photosystem II Reactions. Based on the flash yield for O₂ evolution, it is concluded that the activity of Photosystem II reaction centers is not affected by cation depletion. The effect of divalent actions on O₂ evolution was measured using both concentration and rate electrodes. Earlier, others observed an inhibition in O₂ flash yield by cation depletion, and this inhibition was relieved by addition of MgCl₂. They concluded that Mg²⁺ induced an activation of PS II reaction centers. NRRC researchers have now found that the apparent inhibition in O₂ flash yield is actually due to slow settling of cation-depleted thylakoids on the electrode surface, which results in lower O₂ flash yield. Addition of MgCl₂ enhances O₂ flash yield by greatly increasing thylakoid settling rates but not by activating PS II reaction centers. (See HSC, C.2.)

Double Turnovers in Photoreactions. Characteristics of Photosystem II charge separation and oxygen evolution processes in algae and chloroplasts indicate two types of double hitting or advancement in S-states (forms of energy states by the systems in oxygen evolution: a nonphotochemical type found with flashes as brief as 5 nanoseconds and a photochemical type only seen with microsecond long flashes. The nonphotochemical type is sensitive to physiological condition and is only present in freshly prepared samples. Furthermore, kinetics of the processes involved in double advancement in S-states for oxygen evolution are quite distinct from those involved in double turnovers in charge separation. The former is in a range of microseconds whereas the latter is in a range of tens of nanoseconds. Concepts of the oxygen-yield mechanism are, in addition, simplified by experiments with ferricyanide-treated chloroplasts, which show that one proposed solution to the oxygen S-state distribution is invalid. (See HSC, C.2.)

HPLC System Developed. A study of the greening process using etiolated red kidney beans led to the development of a HPLC system for the determination of protochlorophyllide, chlorophyllide, and intermediates in the production of chlorophyll a. New forms of chlorophyll a, which are important in the early stages of greening, were discovered and partially characterized. (See HSC, C.2.)

Cyclic Acids in Heated Oils. With the increased world consumption of fried foods, there is need to control the safety and any reduction in nutritional quality caused by thermal oxidation of fats. Cooperative research with Professor E. G. Perkins, University of Illinois, led to a convenient gas-chromatographic procedure to determine the amount of cyclic acids in fats heated under commercial frying conditions. The cyclic acids measured by

this method in soybean oils and hydrogenated soybean oils used repeatedly to fry potato slices at 195°C have been identified by gas chromatography-mass spectrometry as C-18 compounds with ortho-disubstituted 6-carbon rings. These biologically active compounds have been found in ranges of 0.3 to 0.6% in heated oils, but the critical level at which they affect the safety of deep-fat fried foods is not known. Diunsaturated cyclic acids of defined structure have now been synthesized by NRRC scientists for biological evaluation at the University of Illinois. [See Oilseed Crops Laboratory (OC), A.2.]

Cyclic Peroxides in Oxidized Oils. Hydroperoxy-cyclic peroxides have been isolated and identified as major secondary products of autoxidized methyl linolenate. Extensive spectral characterization established four isomers with a five-member cyclic peroxide structure and a hydroperoxide positioned either on carbon-9 or carbon-16. These secondary products not only may serve as sources of flavor deterioration in edible fats but also may be involved in biological lipid oxidation. The cyclic peroxides are related to highly physiologically active prostaglandins that are enzymatically formed from arachidonic acid hydroperoxides. Aspects of lipid oxidation that are attracting much attention include damage to membranes, enzymes, and proteins, the problem of aging, and the effect of activated oxygen in environmental pollution. Further research on the biological activity of fatty acid cyclic peroxides is aimed at preventing their formation. (See OC, A.2.)

Hybrid Fuels. As a means of extending farmers' fuel supplies with agriculturally derived products, microemulsions incorporating 190-proof ethyl alcohol into diesel fuel were prepared. One formulation involved an ionic microemulsion involving unsaturated fatty acids and an amine; another involved n-butyl alcohol (also a potential fermentation product) in possibly also a microemulsion system. Engine tests carried out at the University of Illinois indicated little problem with these fuels in the short-term and cleared the way for possible long-term tests. (See OC, B.1.)

Steam Refining Goes Commercial. NRRC investigations demonstrated that pretreatment of crude soybean oil with phosphoric acid is critical to producing quality steam-refined salad oils. Increasing commercial interest in steam refining has been motivated by the need for lower equipment cost, energy conservation, abatement of soapstock pollution from conventional alkali refining, and recovery of valuable fatty acids as byproducts. The NRRC process using a phosphoric acid pretreatment was tested by a major vegetable oil processor. Over 100 tons of crude oil was degummed, bleached, and simultaneously deacidified and deodorized in a continuous steam-refining operation. The resulting bland, light-colored oil was of such high quality that the company is now planning production by this means of another million pounds of oil for test marketing. (See OC, C.2.)

Supercritical CO₂--Petroleum-Free Extraction of Soybeans. Problems with use of hexane for extraction of soybeans include excalating costs, unpredictable supply, and inherent plant hazards involved with the use of a petroleum product. In an effort to find alternatives, a high-pressure gas extractor

was designed to use carbon dioxide above its critical temperature, the temperature above which it may not be liquified regardless of applied pressure. Carbon dioxide is an ideal solvent for edible products because it is nontoxic, readily available, low in cost, nonflammable, nonexplosive, and easily separated from the substrate. Full-fat soy flakes were extracted readily with supercritical CO₂ at a pressure of 5000 psi and 50°C to a residual oil content of less than 0.5%. Oil was separated from the gas phase by reducing the pressure. Solvent-free oil was compared to hexane-extracted oil from the same beans. Because the phosphorus content of CO₂-extracted oil was about one-tenth that of hexane-extracted oil, refining losses should be decreased. There were no other essential differences in chemical analysis. (See OC, C.2.)

Dietary Positional Octadecenoic Acid Content of Human Tissue Lipids. Human heart, brain, liver, aorta, and adipose tissue lipids from subjects ranging in age from 20-80 years were found to contain trans octadecenoic acids isomers having double bond at the 6 through 15 positions. Similarly, cis-octadecenoic acid isomers having double bonds at the 6 through 14 positions were found. No unusually large accumulation of these isomers were found. This is the first analysis of human tissues for geometric and positional fatty acid isomers and provides valuable information on the nutritional impact of hydrogenated vegetable oils on tissue lipids. (See OC, D.1.)

Preparative Separation of Fatty Acids. Chromatographic columns containing an ion exchange resin and silver ions were used to separate mixtures of cis and trans fatty acids and esters having 0 to 3 double bonds. Mixtures of triglycerides containing 0, 3, 6, and 9 double bonds could also be separated. The columns were reusable and have a capacity of 20-40 g.

This achievement fills a general need for a low cost and rapid preparative method for purification of fatty acid isomers from synthetic reactions and for isolation of pure fats from natural products. The technique is versatile and is applicable to a variety of compounds since the separation is based on the number and configuration of the carbon-carbon double bond rather than on gross structural properties. (See OC, D.1.)

Preparation of Deuterium Labeled Fats for Use in Human Studies. The metabolism of the essential fatty acid (linoleic acid) is biologically important because it is the precursor for a variety of compounds associated with the etiology of a number of diseases and disorders afflicting humans.

Several synthetic methods for preparation of deuterium labeled linoleic acid have been evaluated. The best method utilizes an eight-step synthesis and employ the Wittig reaction. This synthetic scheme has been used to prepare sufficient amounts of linoleic acid-d₄ for use in a preliminary human study. Overall yields were 30% and gave a product having 96% isotopic purity. (See OC, D.1.)

Fermentation of Pentose Sugars to Ethanol. Hemicellulose is one of the major components of renewable biomass, comprising up to 35% of plant

materials. Upon hydrolysis, hemicellulose yields a mixture of sugars with D-xylose (a 5-carbon sugar) as the major product. This sugar can be obtained in good yield by means of a simple, low cost, dilute acid hydrolysis at moderate temperatures and atmospheric pressure. However, full utilization of residues for production of fuel and petroleum-sparing compounds requires a new fermentation process to use D-xylose. A systematic search of yeasts from the AR Culture Collection at NRRC has uncovered a yeast that has the unique capability of efficiently converting D-xylose to ethanol. This new yeast is the only organism known to rapidly ferment xylose to alcohol in good yield. The discovery promises a way to produce more alcohol from biomass than heretofore possible and with the techniques and equipment commonly available in the fermentation industry. [See Northern Agricultural Energy Center (NAEC), A.3.]

Energy-Efficient Enrichment of Stillage and Improved Distillers' Grains for Nonruminants. Procedures based on density differences were developed to separate distillers grains to give a fraction that is higher in protein and lower in fiber; this fraction is superior to regular distillers' grains for nonruminant animal feeds. Great savings in energy were achieved by recycling the centrifuged soluble solution from corn stillage into subsequent alcohol fermentations. Alcohol yields were not significantly changed by using the solubles solutions as media for corn fermentation by yeast. (See NAEC, A.4.)

BIOMATERIALS CONVERSION LABORATORY

W. M. Doane, Chief

Research Leaders: R. A. Anderson, M. O. Bagby, R. J. Bothast,
G. E. Hamerstrand, and F. H. Otey

A. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Basic Engineering Studies on Preparation of Soy Foods (R. A. Anderson)

- a. Specific Objective: Determine the viscoelastic properties of soy-based doughs by eccentric rotating disc (ERD) rheometer and relate to properties of extruded food products.

Progress: Improvements were made in methodology for investigating soy dough rheology using a mechanical spectrometer in ERD mode. It was found that mixing time, dough moisture, and percent strain all affected soy dough viscoelasticity. A computer model of soy dough elastic and viscous loss moduli was developed to account for these effects.

- b. Specific Objective: Investigate basic engineering and rheological aspects of extrusion in relation to soy quality in foods.

Progress: Work done previously on the rheological properties of soy dough during extrusion was expanded. The Brabender lab extruder was used with larger dies (3 mm), using varying barrel lengths, feed, and processing conditions. An increase of dough elasticity with increasing extruder residence time was confirmed. Preliminary results confirm that soy dough is shear thinning, similar in nature to thermoplastics. Dough elasticity increased with extruder residence time. The effect of particle size on dough rheology and die pressure drop was studied using soy flour material as feed.

- c. Specific Objective: Establish the relationship between soybean processing variables and the bioavailability of the amino acids of soybean meal in chick feeding studies (cooperative with AES, University of Maryland).

Progress: In the present study, the interrelationship of several factors in the desolvantizing-toasting process for toasting soybean meal and how these relate to protein quality was determined. Independent variables such as moisture, hexane level, temperature, and time were found to be critical factors in the toasting process. These were computer fitted to data which were used to predict several dependent variables such as trypsin inhibitor inactivation, nitrogen solubility index, urease activity, and meal color values.

- d. Specific Objective: Investigate the bioavailability of zinc and other minerals in soy protein diets (cooperative with AES, University of Illinois).

Progress: Soy protein products at three phytate levels in products prepared in the pilot plant were fed to rats at the University of Illinois to evaluate zinc bioavailability. Although the results showed differences between the products, the results were not statistically significant.

- e. Specific Objective: Investigate alternate approaches to the hexane solvent extraction procedures for soybeans.

Progress: The soybean extraction pilot plant at NRRC has been modified to make possible the extraction of soybeans with aqueous alcohols. Additional equipment was installed to coalesce the oil and solvent and yield two separate phases on a continuous basis. Preliminary laboratory experiments were conducted to determine solvent/flake ratio and the rate of buildup of extracted solids and soluble sugars in the recycled alcohol phase. In a continuous pilot plant run at high temperatures, the effectiveness of the equipment was tested and found to give a successful run while evaluating extraction yields and other responses. Studies have also begun on desolventizing-toasting of the extracted meal.

Publications:

BAKER, E. C., G. C. MUSTAKAS, J. W. ERDMAN, JR., AND L. T. BLACK. The Preparation of Soy Products with Different Levels of Native Phytate for Zinc Bioavailability Studies. Accepted for publication in J. Am. Oil Chem. Soc. October 24, 1980.

ERDMAN, J. W., JR., K. E. WEINGARTNER, G. C. MUSTAKAS, R. D. SCHMUTZ, H. M. PARKER, AND R. M. FORBES. Zinc and Magnesium Bioavailability from Acid-precipitated and Neutralized Soybean Protein Products. J. Food Sci. 45(5) (1980):1193-1199.

JASBERG, B. K., G. C. MUSTAKAS, AND E. B. BAGLEY. Comparison of Extrusion and Capillary Flow of Thermoplastics with Soy Dough. Submitted to J. Food Proc. Eng., November 1980.

JASBERG, B. K., N. W. TAYLOR, G. C. MUSTAKAS, AND E. B. BAGLEY. Determination of Dynamic Moduli of Soy Doughs Using an Orthogonal Rheometer. Submitted to J. Text. Studies, November 1980.

MOULTON, K. J., G. C. MUSTAKAS, AND E. C. BAKER. Pilot Plant Desolventizing-Toasting of Extracted Soybean Flakes--A Preliminary Study. Abstract, ISF/AOCS Congress, New York, NY, April 1980.

MOULTON, K. J., G. C. MUSTAKAS, AND E. C. BAKER. Desolventizing-Toasting of Extracted Soybean Flakes--Development of Pilot Plant Equipment and Operational Procedure. Submitted to J. Am. Oil Chem. Soc., February 1981.

MUSTAKAS, G. C., K. J. MOULTON, E. C. BAKER, AND W. F. KWOLEK. Critical Processing Factors in Desolventizing-Toasting Soybean Meal for Feed. Proceedings of World Conference on Soya Processing and Utilization, November 9-14, 1980, Acapulco, Mexico.

2. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn
(R. A. Anderson)

- a. Specific Objective: Reduce energy requirements in the corn milling industry.

Progress: A shortened dry milling flow which eliminates the roller milling and grading steps of the regular flow produced large grits (-3-1/2 + 5) of relatively low fat content (1-3%), but with more attached hulls than grits from normal dry milling operations. In this work, a horizontal solid rotor degerminator was used. As temper time was shortened or temper moisture was lessened to lower energy requirements, attached hull on the grits was increased. While this may be considered undesirable to the breakfast food industry, it should have little effect on usage of these grits for starch or alcohol production.

Preliminary studies on interphased milling, i.e., combination of short-flow dry milling and wet milling, indicate that lower yields of starch might be encountered by these procedures, in which large grits are the raw material. This work is continuing.

The temperature used for drying high-moisture corn has a decided effect on the throughput of stock through the solid rotor degerminator. Throughput of corn dried at 150°C was lowered by up to 20% over the moisture range of 17 to 32% compared to a field-dried control, while corn dried at 49°C had an increased throughput, amounting to as much as 80% over the control at the same range of moistures. As degerminator throughput is increased, horsepower per unit of corn ground is reduced.

Cooperative studies were conducted with USDA Office of International Cooperation and Development (OICD) on the manufacture of prototype products from millet, assisting on a project OICD is involved with in Senegal. Assistance was rendered to the Instituto de Investigaciones Technologicas, Bogota, Colombia, in demonstrating dehulling procedures on two of their native grain sorghums. Procedures were devised for adding ⁶⁵zinc to corn grits in order to provide products for human feeding tests in cooperation with the Cereal Science and Foods Laboratory, NRRC, and the SEA-AR Human Nutrition Laboratory, Grand Forks, ND.

- b. Specific Objective: Evaluate procedures for preparing edible corn germ flour with improved flavor and shelf life.

Progress: Quick tempering (20%) full-fat dry-milled germ permits separation of bound lipids and other bitter constituents by liquid classification (simulated cyclone process) during oil recovery. These off-flavor germ components are emulsified and removed with residual hull and endosperm contaminants. Preliminary tests indicate that taste and shelf life of these protein flours are improved when compared to flours obtained by standard processing. Flours with high levels of lysine and sulfur amino acids can be fractionated by this process.

- c. Specific Objective: To adapt present dry-milling methods to the milling of high-oil corn mutants.

Progress: Standard dry-milling procedures even with a higher than normal temper does not contain the oil within the germ during milling of high-oil corn mutants. Thus, the oil contaminates the grits and meal fractions, reducing their shelf life and food quality. Microscopic examination of tissue sections of these mutants transected through the germ shows a microstructure similar to that of normal corn except that the oil-containing particulates are swollen with oil and rupture during degeneration. Degermination is not recommended for milling high-oil corn mutants.

- d. Specific Objective: To develop less energy-intensive milling processes for the production of starch or low-protein flours.

Progress: In developing a less energy-intensive process for isolation of starch, various corn genotypes that could be field grown economically were examined. Hybrid studies were done on opaque-2 parented from normal and waxy corns. These hybrids have a dense horny peripheral endosperm layer, which provides protection in handling and storage similar to that of normal dent corn. Microstructure of the protein and starch within most of the endosperm cells of the opaque-2 shows looser organization than normal dent corn, which affords simpler methods to isolate low-protein flours. Preliminary tests show more rapid hydration and chemical digestion of protein during steeping.

- e. Specific Objective: To study protein-starch separation of endosperm as a function of grit fractions obtained by interphase milling in order to obtain a more efficient process.

Progress: The initial stages of the steeping process were studied in detail in an effort to reduce process energy needed for production of corn starch. Degerminating corn prior to steeping improved rate of hydration of the endosperm fraction (grits, meal, and flour). This hydration, coupled with increased fermentation by

Lactobacillus, softens the horny portion of the endosperm, and particles can be wet ground to finer size. The increased surface area reduced residence time in SO₂ during the final stages of steeping. Scale-up studies are needed to assess economic feasibility of these adaptations for processing corn starch.

Publications:

CHRISTIANSON, D. D. Food-Grade Protein Concentrates from Dry-Milled Yellow, White, and High-Oil Corn Germ. For Cereal Foods World. In preparation.

NAVICKIS, L. L., R. A. ANDERSON, AND E. B. BAGLEY. Experiments with Wild Rice--Subject of Illinois Report. Rice J. 83(6) (1980):9-13.

PEPLINSKI, A. J., D. D. CHRISTIANSON, AND R. A. ANDERSON. Note on the Dry-Milling of High-Oil Corn. For Cereal Chemistry. In preparation.

Other Reports:

BOTHAST, R. J., R. A. ANDERSON, K. A. WARNER, AND W. F. KWOLEK. Effects of Moisture and Temperature on Microbiological and Sensory Properties of Wheat Flour and Corn Meal During Storage. Presented by R. A. Anderson at the 65th Annual Meeting of the American Association of Cereal Chemists, September 1980, Chicago, IL. Submitted to Cereal Chem., October 1980.

CHRISTIANSON, D. D. Energy Conservation and New Corn Foods by Interphase Milling. Presented at the 21st Annual Dry Milling Conference, Peoria, IL, June 1980.

CHRISTIANSON, D. D., H. YOSHIKAWA, K. WARNER, L. H. KRULL, AND G. E. INGLETT. Food-Grade Protein Concentrates from Dry-Milled Yellow, White, and High-Oil Corn Germ. Presented at the 65th Annual AACC Meeting, Chicago, IL, September 1980.

PEPLINSKI, A. J., S. R. ECKHOFF, K. A. WARNER, W. F. KWOLEK, AND R. A. ANDERSON. Dry Milling of Preserved High-Moisture Corn. Presented at the 65th Annual Meeting of the American Association of Cereal Chemists, September 1980, Chicago, IL.

3. Development of Mycostatic Systems to Permit Safe, Low-Energy Grain Drying (R. A. Anderson)
 - a. Specific Objective: Secure Environmental Protection Agency (EPA) approval for preservation and ambient air drying of high-moisture corn.

Progress: EPA clearance for use of sulfur dioxide as a fungicidal agent to retard mold growth on high-moisture corn while drying grain with ambient air was granted and published in the Federal Register on June 19, 1980. Approval requires that treated corn be for feed use only.

Publications:

ECKHOFF, S. R., J. E. VAN CAUWENBERGE, R. J. BOTHAST, G. W. NOFSINGER, AND E. B. BAGLEY. Sulfur Dioxide-Supplemented Ambient Air Drying of High-Moisture Corn. Trans. ASAE 23 (1980):1028-1032.

MILLER, D. L. Trickle Ammonia Process--Preservation of Corn While Drying with Ambient Air. Proceedings 1980 Grain Conditioning Conference, University of Illinois, Urbana-Champaign, IL, January 30-31, 1980:51-55.

NOFSINGER, G. W., J. E. VAN CAUWENBERGE, R. A. ANDERSON, AND R. J. BOTHAST. Preliminary Biological Evaluation of the Effect of Microwave Heating on High-Moisture Shelled Corn. Cereal Chem. 57 (1980):373-375.

VAN CAUWENBERGE, J. E., AND R. J. BOTHAST. A Comparison of Controlled-Release Ammonia Solutions with Aqueous Ammonia for Preserving High-Moisture Maize. Accepted for Publication in Cereal Chemistry.

B. TECHNOLOGIES FOR INDUSTRIAL USES OF
PLANT AND ANIMAL PRODUCTS

1. Starch-Based Copolymers for Making Elastomers (T. P. Abbott)

- a. Specific Objective: Determine if a deionized commercial styrene-butadiene-vinyl pyridine polymer combined with a deionized sodium carboxymethyl starch will produce an ionically bonded reprocessable composite.

Progress: Combinations of the above-described polymer and starch were tested and found to have tensile strengths not significantly higher than the unreinforced elastomer.

Publications:

ABBOTT, T. P. AND C. JAMES. Grafting of 2-Butenyl Acrylate on Starch. J. Appl. Polym. Sci. In press.

2. Starch-Based Purification Aids for Wastewater (W. M. Doane)

- a. Specific Objective: Complete work on starch-based products for removing heavy metals from wastewaters.

Progress: Several procedures were developed to attach polyethylenimine onto crosslinked starch. Evaluation of the synthesized products showed excellent copper and metal cyanide removal from industrial wastewaters.

All the starch-based products developed in this program were evaluated as ion exchange materials in paper. Handsheets were prepared containing either the anionic or cationic starch-based ion exchange products. Wastewaters containing heavy metal ions were treated with the ion exchange paper and excellent removal resulted.

Publications:

RADOSEVICH, J. A. AND R. E. WING. Starch-based Ion Exchange Materials in Paper: Preparation and Use in Heavy Metal Removal. *Staerke* 32 (1980):424-428.

RAYFORD, WARREN E. AND ROBERT E. WING. Crosslinked Starch Halohydrins and Their Nitrogen-containing Substitution Products. U.S. Patent 4,237,271. December 2, 1980.

WING, R. E. Electroless Nickel Rinse Waters are Treatable. Proceedings of the Electroless Nickel Conference, Cincinnati, OH. 1979.

WING, R. E. Emerging Treatment Technologies in Removing Metals. American Eletroplaters Society Illustrated Lecture in "The AES Training Course for Water Pollution Control and Compliance." 1980.

WING, R. E. Treatments for Complexed and Chelated Rinsewaters. American Electroplaters Society Illustrated Lecture in "The AES Training Course for Water Pollution Control and Compliance." 1980.

WING, R. E. AND W. E. RAYFORD. Polyethylenimine-starch Products: Preparation and Use in Heavy Metal Removal. *Staerke* 32 (1980):299-302.

WING, R. E. AND W. M. DOANE. Wastewater Treatment of Acid Cadmium Rinses. *Plat. Surf. Finish.* 68 (1981):50-54.

3. Flame-Resistant Polyurethane Foams and Biodegradable Films and Packaging Prepared from Starch (F. H. Otey)

- a. Specific Objective: Evaluate the mechanical properties and extrusion blowing characteristics of compositions having biodegradable properties.

Progress: Earlier studies revealed that quality films can be extrusion blown from various combinations of gelatinized starch, poly(ethylene-co-acrylic acid) (EAA) and, optionally, polyethylene

(PE). More extensive studies on the preparation and evaluation of the films were completed. Films made with 10 to 50% starch, with EAA being the remaining composition, had tensile strengths of 3,470 to 3,940 psi and percentage elongations of 260 to 61. Films made with 40% starch and up to 50% PE, used as a replacement for part of the EAA, had decreasing tensile strength from 3,570 psi to 1,840 psi and percentage elongations from 80 to 10% as the PE was increased from 10 to 50%. While starch-EAA alone gave films with better mechanical properties, the preferred formulation was about 40% starch, and 30% each of EAA and PE because of the lower cost of PE.

Films with good physical properties and increased rates of fungi attack were obtained by incorporating various levels of polyvinyl alcohol or sorbitol.

Properties and economics of these films suggest that they could have application as biodegradable mulch film.

- b. Specific Objective: Continue a systematic approach to find an optimum method for plasticizing starch film with the ultimate aim of establishing a correlation between the chemical structure of polyols and other classes of organic compounds and their effectiveness as plasticizers for starch films.

Progress: The formate ester precursors of 1,2,3-butane triol, 1,2,3-pentane triol, 1,2,4-pentane triol, 1,2,5-pentane triol, and 2,3,4-pentane triol have been synthesized and are ready for de-esterification to the desired free triols needed to complete the polyol film plasticizing study. While the esterification step did not seem to offer any problems, an attempted de-esterification of the 1,2,3-butane triol ester did not go smoothly to the free polyol. Infrared spectral absorption studies and elemental analyses indicated that some unsaturated starting material had remained in the ester formed and carried over through the de-esterification. This difficulty will be alleviated in the continued syntheses with alterations in the somewhat standardized procedures as needed.

Publications:

MAHER, G. G. Foamed and Solid Rubber-graft Copolymer Compositions and Method of Preparation. Canadian Patent 1,089,575. November 11, 1980.

OTEY, F. H., R. J. WESTHOFF, AND W. M. DOANE. Starch-Based Blown Films. Ind. Eng. Chem., Prod. Res. Dev. 19 (1980):592-596.

4. Controlled Release of Pesticides by Formulating with Cereal-Based Starch and Flour Derivatives (W. M. Doane)

- a. Specific Objective: Evaluate starch-alkaline earth adducts as potentially new encapsulating agents for pesticides.

Progress: A new method was evaluated for encapsulating pesticides which does not require the toxic and highly flammable CS₂ reagent. The method consists of mixing pesticides with alkali starch followed by precipitation with alkaline earth salts. Using trifluralin as the active agent, variations in the encapsulating procedure that were investigated and found to influence pesticide encapsulation included pesticide loading, starch solids concentration, ratios and amounts of sodium hydroxide and alkaline earth salts, and mesh size of the final product. Although the Ca adduct was slightly more effective in retaining pesticide than Sr and Ba adducts at the preferred 2/1 NaOH/MCl₂ molar ratios, both of the latter two adducts gave reasonably high levels of encapsulation. When 1.3 to 20 g of trifluralin was used per 45 g of starch, about 90% of the pesticide was completely encapsulated. Scanning electron microscopy examination of the dried precipitates revealed that the pesticide was entrapped in small cells within the starch-calcium adduct. The scope of the new procedure was demonstrated by encapsulating 20 other pesticides. Even volatile pesticides were contained by the starch matrix.

- b. Specific Objective: Continue to cooperate with AR and university agronomists on evaluation of starch-encapsulated pesticides.

Progress: NRRC chemists prepared several new starch-encapsulated pesticides for greenhouse and field testing by cooperating scientists. So far, cooperative work with academia has resulted in four masters and two doctors theses and two more graduate studies are nearing completion. At the University of Illinois, V. Raboy found that the release rate of encapsulated chloramben varied greatly depending upon the encapsulating techniques used by the NRRC chemists. Raboy also found that the persistence of chloramben acid and its methyl ester is improved by encapsulation. Field or greenhouse studies were conducted with NRRC products by: M. Schreiber, SEA-AR scientist at Purdue University with butylate, EPTC, and trifluralin; B. Hoffman at the SEA-AR Beltsville Agricultural Research Center with trifluralin and profluralin; and B. Devisetty at Hopkins Agricultural Chemicals Company with trifluralin. Each study demonstrated that starch encapsulation improved the performance of these pesticides.

- c. Specific Objective: Evaluate decomposition and release of certain encapsulated pesticides.

Progress: The stability of diazinon encapsulated in crosslinked starch xanthate was determined in accelerated aging tests performed at 70°C and related to preparative variables of encapsulation. Instability of diazinon discovered under these conditions was related to the formation of acidity in the capsules. The diazinon in the capsules was successfully protected from decomposition by adding calcium oxide.

The release of trifluralin from several formulations was induced using water and measured by techniques of volatilization into filter paper and desorption into organic solvents.

- d. Specific Objective: Laboratory evaluation of starch esters of herbicides as slow release agents.

Progress: Starch-bonded picloram containing 39.4% active agent was tested in sterilized and nonsterilized soil using lettuce as the target plant. Results showed that microorganisms of nonsterile soil were necessary for herbicide release. Rate of release was dependent on hydrolysis time in the presence of nonsterile soil.

Publications:

HERBERT, E. W., H. SHIMANUKI, AND B. S. SHASHA. Brood Rearing and Food Consumption by Honeybee Colonies Fed Pollen Substitutes Supplemented with Starch-encapsulated Pollen Extracts. *J. Apic. Res.* 19(2) (1980):115-118.

HERBERT, E. W., H. SHIMANUKI, AND B. S. SHASHA. Encapsulation of Pollen Attractants for Honeybee, Aris mellifera L. Diets. *New York Entomological Society* 88 (1979):73-76.

McGUIRE, T. A., R. E. WING, AND W. M. DOANE. Preparation of Starch Esters of Herbicides and Their Evaluation as Slow Release Agents. *Staerke* (1980). In press.

SHASHA, B. S. Starch and Other Polyols as Encapsulating Matrices for Pesticides. In Controlled Release Technologies: Methods, Theory, and Applications. Vol. II. Edited by Agis F. Kydonieus, CRC Press, Inc., Florida. 1980. Pages 207-223. Book chapter.

TRIMNELL, D., B. S. SHASHA, AND W. M. DOANE. Degradation of Diazinon Encapsulated with Starch Xanthate. *J. Agric. Food Chem.* 29 (1981):145-148.

5. Graft Polymers of Starch for Agricultural Chemicals and Absorbents (G. F. Fanta)

- a. Specific Objective: Study the graft polymerization onto wheat straw biomass.

Progress: The polymer fraction isolated from the sodium hydroxide extraction of wheat straw (hemicellulose plus lignin) was subjected to graft polymerization with acrylonitrile using ceric ammonium nitrate initiation. There was no conversion of acrylonitrile to polymer. Since it was suspected that the lignin component might be inhibiting polymerization, lignin was extracted by the chlorous acid procedure and the graft polymerization was repeated on the resulting hemicellulose fractions. There was still no conversion of acrylonitrile to polymer. The small amounts of lignin still present after chlorous acid delignification might be sufficient to inhibit polymerization; however, other components of this hemicellulose fraction could also be acting as inhibitors. Removal of extraneous salts and low molecular weight carbohydrates by dialysis against distilled water did not improve reactivity.

- b. Specific Objective: Study the graft polymerization onto hemicellulose.

Progress: Ceric-initiated graft polymerizations onto a commercial sample of larch wood hemicellulose were studied. Heating the hemicellulose-water slurry to 85°C before room-temperature graft polymerization with acrylonitrile gave a product which was not greatly different from that produced in the absence of the preheating step. Over half of the graft copolymer could be removed by successive room-temperature extractions with dimethylformamide (DMF) and dimethylsulfoxide (DMSO). DMF-soluble fractions contained about 70-75% polyacrylonitrile (PAN), while the remaining polymer contained 40-50% PAN. Molecular weights for PAN were in the 100,000 range. Graft polymerization of methyl acrylate gave a product containing about 40% poly(methyl acrylate) after removal of homopolymer by acetone extraction. This hemicellulose-g-poly(methyl acrylate) could be extrusion processed to form a leathery, flexible plastic with a tensile strength (UTS) of about 3,200 psi.

- c. Specific Objective: Study the crosslinking mechanism which occurs when acrylonitrile is first polymerized within a polysaccharide matrix and then saponified.

Progress: Removal of the starch portion of saponified starch-g-polyacrylonitrile (PAN) by enzymatic hydrolysis yielded saponified PAN which was not crosslinked and which was about the same molecular weight as its PAN precursor. PAN-PAN crosslinking during aqueous alkaline saponification is, therefore, not occurring. If the saponification is conducted in ethanol-water, however, PAN-PAN crosslinking does take place when ethanol is the major component of the ethanol-water solution. One of the reactions leading to crosslinking in saponified starch-g-PAN takes place between starch and PAN during alkaline saponification. Samples of starch-g-PAN, which were soluble in dimethylsulfoxide (and thus not crosslinked),

gave aqueous dispersions on saponification that contained measurable amounts of highly swollen, insoluble gel. Although starch and PAN gave noncrosslinked solutions when saponified individually, a physical mixture of the two also produced a viscous dispersion containing insoluble gel--an indication of an alkaline-induced reaction between the two components. Another reaction which leads to crosslinking takes place during the graft polymerization reaction, and this reaction path probably involves the chain combination of a small percentage of growing PAN macroradicals. Low solubilities in dimethylsulfoxide were observed for starch-g-PAN copolymers; however, solubilities increased when graft copolymers were prepared in the presence of chain transfer agents.

- d. Specific Objective: To better understand the mechanism of water swelling of hydrolyzed starch-graft copolymers and other gels, measurements will be continued of the rheological properties of model gels containing carboxamide and carboxylic acid functional groups.

Progress: The procedure developed for this study employed stress relaxation of the gels in a controlled humidity chamber on a mechanical spectrometer. The method gave satisfactory data for high viscosity gels, but most gels of interest have moduli below the range of the spectrometer. Although the method was especially useful for separating the polymer-entanglements component from the permanent-crosslinks component of the elastic modulus, this objective was abandoned for lack of a more sensitive rheometer.

- e. Specific Objective: Collect and assimilate background information on the addition of organic materials to poorly structured soils for improvement or "conditioning" of the soil; and adapt or develop a screening test that, using small soil samples, will reliably measure the relative water stabilization of soil aggregates when test materials are applied, thus providing a guide for chemical modification.

Progress: There is a long list of physically and chemically active materials which can be added to the soil to improve its physical condition (i.e., control soil washing or blowing, improve soil drainage, improve air-water relations in soils, overcome or induce water repellancy, etc.). Two effective materials for stabilizing soil aggregates to water are polyvinyl alcohol (PVA) solutions and bituminous emulsions. Their advantages are that they can be spray applied to prepared soil surfaces and they are effective at low levels of application. Drawbacks to these materials include cost, decreasing supply, and handling difficulties. Chemical similarity of the synthetic polymer (PVA) to the naturally occurring polymer starch led to preliminary investigations which showed starch to have very little aggregate stabilizing ability, whereas certain modified starches, such as some starch ethers, are almost as effective as PVA.

The screening test now in use requires the placing of 25 g samples of dry, size-graded soil particles in petri dishes, droplet or small stream application of dispersions of test polymers, air drying, and wet sieving using a nest of four screens (5, 3, 1, and 0.5 mm). Tests are run in duplicate, and a concentration curve is obtained by applying dispersions of at least three different concentrations and plotting concentration against percent of dry soil retained by screens. Effects of varying degrees and types of modifications are compared by comparing concentration curves.

- f. Specific Objective: Examine the extrusion of carbohydrate-poly(methyl acrylate) graft copolymers and study the physical properties of the resulting plastics.

Progress: Master batches of starch-poly(methyl acrylate) copolymers have been polymerized under a variety of reaction conditions, freed of poly(methyl acrylate) homopolymer, recovered, and characterized preparatory to physical manipulations on tests.

Publications:

FANTA, G. F., R. C. BURR, AND W. M. DOANE. Polymerization of Alkyl Acrylates and Alkyl Methacrylates with Starch. *J. Appl. Polym. Sci.* 25 (1980):2285-2294.

FANTA, G. F., R. C. BURR, W. L. ORTON, AND W. M. DOANE. Liquid-Phase Dehydration of Aqueous Ethanol-gasoline Mixtures. *Science* 210 (1980):646-647.

TRIMMELL, D. AND E. I. STOUT. Grafting Acrylic Acid onto Starch and Poly(vinyl Alcohol) by Photolysis. *J. Appl. Polym. Sci.* 25 (1980):2431-2734.

Patents: FANTA, G. F., E. I. STOUT, AND W. M. DOANE. Highly Absorbent Polyhydroxy Polymer Graft Copolymers Without Saponification. U.S. Patent 4,194,998. March 25, 1980.

Other Reports:

FANTA, G. F. Preparation and Properties of Saponified Starch-g-Polyacrylonitrile (Super Slurper). Presented at the 73rd Annual Meeting of AIChE, Chicago, IL. November 18, 1980.

6. Plant Component Separation and Physical Characterization (T. P. Abbott)

- a. Specific Objective: Initiate new studies on methods and mechanisms to modify biopolymers to provide an expanded knowledge base for replacing petroleum-derived polymers with natural polymers.

Progress: The lignin literature was studied in depth, and areas for research were evaluated. A decision was made to investigate the reaction of acetylene with lignin which is not well understood, in order to obtain an energy-efficient reaction to modify the natural polymer.

- b. Specific Objective: To determine the nature of biodegradation products of lignin from Cyathus stercoreus fermentation of wheat straw.

Progress: Analytical methods were developed to separate several biodegradation products of lignin reported in the literature. The methods were tested with an oxidatively degraded wheat straw lignin and found to be reliable. Analytical protocol for isolating biodegradation products from fermentations was developed.

7. Basic Studies on Modification of Natural Polymers as Replacements for Petroleum-Derived Polymers (F. H. Otey)

- a. Specific Objective: Initiate research on chemical and physical modifications of biopolymers and determine effects and mechanisms of these modifications to provide an expanded knowledge base for replacing petroleum-derived polymers with natural polymers, especially in thermoplastics.

Progress: Earlier studies demonstrated that unmodified natural polymers can be blended with synthetic polymers to yield biodegradable plastics. Studies have now been approached to modify natural polymers, both chemically and physically, in an effort to improve flexibility and compatibility. Preliminary results suggest that the incorporation of various ether groups onto natural polymers may achieve the desired results.

- b. Specific Objective: Initiate studies on the gelatinization of natural polymers as a method for improving their application in films.

Progress: A basic study was initiated on the alkali-gelatinization of starches that are representative of current commercial modified starches. Data were generated on the amount of alkali needed for complete gelatinization, on the viscosity behavior of the ensuing gels and syrups, and on visual and microscopic characteristics at the various stages of gelatinization. This study is expected to provide useful information for film preparation.

8. Basic Studies on Biopolymers for Improving Safety of Pesticides (F. H. Otey)

- a. Specific Objective: Initiate chemical and physical studies on methods and mechanisms of pesticide entrapment with modified

biopolymers to expand the knowledge base for improving the safety of pesticide application.

Progress: Studies were initiated to standardize procedures for encapsulating pesticides by the biopolymer xanthate procedure. Studies were approached to include safety during production, testing, and use. Preliminary results show that quantities of chemicals, temperature of reaction, and rate of mixing are critical to optimize encapsulation.

A search for new technologies to encapsulate pesticides was begun. Preliminary research was initiated for a tank mix system that would slow the release of volatile pesticides. Initial contacts were made with agencies outside NRCC to run toxicological studies on encapsulated insecticides in comparison with commercial formulations.

Analytical methods were set up for assaying pesticides used at field levels in order to validate formulations for the slowing of pesticide release in tank mix applications.

Several indicators were incorporated into starch xanthide-encapsulated diazinon and found to be feasible for showing decomposition of diazinon through color changes during accelerated aging tests. Such indicators should be useful for detecting shelf life of these products.

- b. Specific Objective: Initiate studies to formulate mathematical expressions for the absolute rate of dissolution and/or diffusion of particular natural-polymer encapsulated pesticide compositions to provide a basis for understanding the relationship between composition variables and release rate of the pesticide.

Progress: Through literature studies, some theoretical principles employed in the design and evaluation of sustained release systems for pharmaceuticals have been found to be conceptually applicable to the mechanism of pesticide release from polymeric systems. Mathematical models based on Fick's law of diffusion are being studied which, with some modification, should provide the framework for a physical interpretation of pesticide release from biopolymeric matrices.

- c. Specific Objective: In order to match the specific requirement of a particular crop, techniques will be evaluated for mixing slow-release pesticide systems of different chemical compositions and particle size distributions so that the pattern of release changes with time.

Progress: Computer models based on semiempirical equations and multiple linear regression have been derived that provide a technique for optimizing controlled-release pesticide formulations to approximate any desired release-rate pattern.

9. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. O. Bagby)

See Northern Agricultural Energy Center, A.1.

10. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. J. Bothast)

See Northern Agricultural Energy Center, A.2.

11. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

See Northern Agricultural Energy Center, A.3.

C. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. Principles Underlying Design of Food Blends for the Export Market (R. A. Anderson)

a. Specific Objective: Continue development and evaluation of new cereal food products with improved nutritional quality.

Progress: The testing of added tricalcium phosphate (TCP) for compatibility with various PL-480 commodities is continuing. Results indicate that insect infestation is being inhibited in bulgur, soy-fortified bulgur, soy-fortified rolled oats, soy-fortified sorghum grits, soy-fortified bread flour, and soy-fortified corn meal, each containing 1 and 2% TCP.

Studies on the use of microwave energy to destroy salmonella in corn-soy-milk (CSM) indicate the practicality of this methodology. Treatment of CSM at a maximum temperature of 67°C in a microwave tunnel followed by cooling to 43°C within 24 hours reduced Salmonella senftenberg from 400 cells/g to 1 cell/g with minimum changes in nutritional quality.

When corn-soy-milk was exposed to high temperatures, various degrees of nonenzymatic browning occurred. Product color ranged from yellow to dark brown. Protein quality was estimated by protein efficiency ratio (PER), Tetrahymena pyriformis W bioassay, essential amino acid analysis, and available lysine values. Color measurements were also made using the Hunter Colorimeter. Hunter "L" values (light to dark) provided a rapid method for predicting protein quality. Correlation with PER was 0.95 using Hunter "L" and 0.88 using available lysine values. This methodology was utilized in subsequent research and proved to be extremely reliable, in addition to saving both time and the costs of PER assays.

Studies were carried out to develop methodology for predicting protein quality relative to cost by graphical methods developed by

computer programming. The method was used to formulate corn-soy based food blends that were low in cost and high in quantity and quality of protein. Triangular coordinates were used to represent a three-component system of nonfat dry milk, peanut flour, and whey protein concentrate. Ratios of chemical score to cost indicated that formulations containing large amounts of corn meal and soy flour were most cost efficient.

- b. Specific Objective: Investigate extrusion as a means of altering structural properties of cereal and cereal products and relate to changes in functional and nutritional properties of cooked cereals in food blends.

Progress: Basic rheological studies on five wheat flour doughs started in 1979 were completed, establishing viscoelastic behavior using the mechanical spectrometer. Flour doughs from different wheat types, prepared in such a manner that the resulting doughs had the same consistency, differed rheologically. Flour doughs prepared at different moisture levels (35 to 47%) from the 5 wheat types without regard to consistency, exhibited large spreads in the elastic (G') and viscous (G'') moduli between types as moisture levels were increased. This behavior is thought to be due to more water being available to swell the starch. In contrast to wheat gluten-water systems, G' and G'' for the wheat flour doughs did not vary with sample thickness. A mathematical model was formulated relating G' of the wheat flour doughs with moisture, protein, and shear rate. An electro-mechanical integrator was designed for the Brabender Plasticorder/Farinograph. This instrument permits accurate known input of work-energy to be imparted to any mixing or extrusion operation utilizing the Brabender apparatus.

Publications:

BOOKWALTER, G. N., R. J. BOTHAST, W. F. KWOLEK, AND M. R. GUMBMANN. Nutritional Stability of Corn-Soy-Milk Blends After Dry Heating to Destroy Salmonellae. *J. Food Sci.* 45 (1980):975-980.

BOOKWALTER, G. N. AND W. F. KWOLEK. Predicting Protein Quality of Corn-Soy-Milk Blends after Nonenzymatic Browning. *J. Food Sci.* Accepted for publication.

Other Reports:

BOOKWALTER, G. N. AND W. F. KWOLEK. Predicting Protein Quality of Corn-Soy-Milk after Nonenzymatic Browning. Presented at the annual meeting of the Institute of Food Technologists, New Orleans, Louisiana, June 1980.

BOOKWALTER, G. N. Research on Food for Peace Commodities. Presented at the World Conference in Soya Processing and Utilization, Acapulco, Mexico, November 1980.

BOOKWALTER, G. N. Soy-Fortified Food Mixtures. Presented at the Human Nutrition Center, Mexico City, Mexico, November 1980.

CEREAL SCIENCE AND FOODS LABORATORY

G. E. Inglett, Chief

Research Leaders: E. B. Bagley, F. R. Dintzis, and J. S. Wall

A. TECHNOLOGIES FOR FOOD AND FEED
USES OF FIELD CROPS

1. Interactions of Food Carbohydrates (E. B. Bagley)

- a. Specific Objective: Determine the mineral ion binding properties of the water-soluble and water-insoluble fractions of hard red spring wheat bran and compare results with data of the soft white wheat bran already analyzed.

Progress: The methodology for determining total bile acid by enzymatic oxidation and individual bile acids in bile acid mixtures by GLC was developed. Although the basic enzymatic method was described by Iwata and Yamasaki, the appropriate reaction conditions had to be chosen to ensure reliable and reproducible results. In the GLC work, it was discovered that quantitative results in a mixture of unconjugated bile acids were possible without resorting to trifluoroacetylation. Silyl derivatives of bile acids were easily but slowly (3 hours) separable on a 22-ft GLC column.

- b. Specific Objective: Determine the fractions of hard red spring wheat that are active in forming complexes with cholesterol and bile acids to specify the source of the hypocholesterolemic effect noted for this bran and not for other brans.

Progress: Preliminary studies of bile acid binding were conducted with AACC soft white wheat bran in order to provide basic information necessary for the study of interactions of bile acids with hard red winter wheat bran. The water-soluble glyco conjugates of the three major bile acid salts in the human intestine (glycocholate, glycocodeoxycholate, and glycochenodeoxycholate) were investigated. At the level of bile acid concentration normally found in the intestine ($\sim 0.008M$), considerable complexation occurs, the degree of interaction increasing in the order glycocholate < glycocodeoxycholate < glycochenodeoxycholate. The extent of interaction is a function of bran concentration and ionic strength. Eight grams of bran forms an insoluble complex with 70% of the glycochenodeoxycholate anions in 1 liter of $0.008M$ sodium glycochenodeoxycholate at 37°C and at an ionic strength of 0.16. Present data suggest that soluble bile acid complexes are also formed, but formed to an extent less than that of the insoluble complexes. Further experimentation will be needed to prove or disprove the presence of soluble complexes.

Publications:

CHRISTIANSON, D. D., J. E. HODGE, D. OSBORNE, AND R. W. DETROY. Gelatinization of Wheat Starch as Modified by Xanthan Gum, Guar Gum, and Cellulose Gum. Submitted for publication in Cereal Chemistry.

RENDLEMAN, J. A. Cereal Complexes. Binding of Calcium by Bran and Components of Bran. Submitted for publication in Cereal Chemistry.

RENDLEMAN, J. A., AND C. GROBE. Cereal Complexes. Binding of Zinc by Bran and Components of Bran. Submitted for publication in Cereal Chemistry.

2. Corn Starches--Physical Characteristics and Biological Digestibilities (F. R. Dintzis)

- a. Specific Objective: Complete study of enzymatic digestibility of starch granules of various sizes from three commercial corn types.

Progress: The α -amylase digestibility of Amylomaize V granules was found to vary with granule size in a manner similar to that of Amylomaize VII, i.e., granules of intermediate size were more resistant to enzyme attack than either the largest or the smallest granules. Enzyme susceptibility in normal dent corn starch varies in direct proportion to the surface area. This pattern is observed also in the amyloazines but is complicated by the variation in amylose content of these samples; increased amylose content is accompanied by decrease in enzyme digestibility. A slight tendency for reduced digestibility of gelatinized starch from intermediate size granules with β -amylase was also observed, but the variation was too small to be significant with respect to determining any differences in the degree of branching of the fractions.

- b. Specific Objective: Study the composition, properties, and physical characteristics of starches from single and double mutant varieties of corn.

Progress: A mercuric chloride steep procedure for extraction of starch from kernels was evaluated for its usefulness in this work. Refinements in the procedure were accomplished to the point that samples were obtained with less granule damage than occurs with SO_2 steep, with acceptable levels of residual protein and endosperm fragments. The procedure is still limited in usefulness for quantitative study of α -amylase digestibility because of inactivation of the enzyme if any residual mercury is present.

A method has been developed whereby the morphology, birefringence, staining properties, and digestibility of individual starch granules can be studied under the light microscope at various stages of

enzyme treatment. Differences were apparent in the rate of enzyme digestion of granules with different staining properties. Variations were also seen between mutants studied.

The use of differential scanning calorimetry as a means of determining gelatinization behavior was investigated. In amyloamaizes, considerable variation in enthalpy and temperature of gelatinization occurs among granules of different size. Lipid content and the amount of water added to the sample also affect the gelatinization. The method appears to be very useful for evaluating starches of different types.

Publications:

CLUSKEY, J. E., C. A. KNUTSON, AND G. E. INGLETT. Fractionation and Characterization of Dent Corn and Amylomaize Starch Granules. *Staerke* 32 (1980):105-109.

KNUTSON, C. A., J. E. CLUSKEY, AND F. R. DINTZIS. Properties of Amylose-Iodine Complexes Prepared in the Presence of Excess Iodine. Submitted for publication in Carbohydrate Research.

3. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn (E. B. Bagley)

See Biomaterials Conversion Laboratory, A.2.

4. Interactions of Dietary Fibers from Cereal Products with Mutagens in Digested Food (F. R. Dintzis)

a. Specific Objective: Determine whether any of the different types of dietary fiber in controlled human diets are instrumental in removing potential carcinogens from the digestive tract.

Progress: In coordination with the University of Illinois Environmental Research Laboratory, Ames Test for mutagens was performed on the extracts from 14 fecal samples. Three dosage levels were used with both TA-98 and TA-100, with and without S-9 extract. These tests indicated unequivocal positives: 0; suspected positive: 2; negative: 10; toxic: 2. Three rats were injected with Arochlor 1254 and sacrificed 5 days later. The livers were excised and treated as described by Ames Test for the preparation of S-9.

Publications:

LEHRFELD, J. A Versatile and Inexpensive Ebullator for Sample Concentration. *J. Chem. Educ.* 57 (1980):587.

LEHRFELD, J. Differential Gas-Liquid Chromatography Method for Determination of Uronic Acids in Carbohydrate Mixtures. Submitted to Anal. Biochem.

5. Methods of Analysis to Facilitate Genetic Improvements of Cereal Grain Protein (J. S. Wall)

- a. Specific Objective: Determine genetic relationships of cereals and their proteins through amino acid sequence analyses.

Progress: Additional amino-terminal amino acid sequence information has been obtained for prolamines from barley, corn, teosinte, and sorghum. These data have been assembled and compared with previously obtained data on amino acid sequences of prolamines from wheat. Evidence for the genetic relationships of cereal grains based on prolamine sequences supports widely accepted concepts which are derived from studies of morphology and generic interbreeding.

Amino-terminal sequence analyses of three isolated water-soluble, alcohol-soluble, reduced corn glutelin polypeptides have established the sequence NH₂-Thr-His-Thr-Ser-Gly-Gly-Cys-Gln-Pro-(Pro-Pro-Pro-Val-His-Leu)₅₋₇ for each protein, with significant homology between them. This protein has a higher proline content than any known previously examined plant protein; its high histidine content explains its dual solubility in water and ethanol. A computer search has failed to detect the sequence -Pro-Pro-Pro-Val-His-Leu- in any other protein.

Four purified 19,000 Mr corn zein samples, some of which were nearly homogeneous, were subjected to NH₂-terminal sequence analysis as the first step in obtaining more extensive sequence information for these proteins. Primarily, a single sequence was obtained for each protein, and marked homology was noted. Initial trials of peptide sequencing using the sequencer with Polybrene added to the samples were successfully completed.

- b. Specific Objective: Analyze genetic control of wheat proteins by improved electrophoretic and chromatographic methods as a basis for breeding varieties having better quality.

Progress: A method for isolating and analyzing gliadin and glutenin from a single wheat kernel was further developed. It consists of (1) removing germ (for further propagation), (2) grinding, (3) extracting gliadin with aluminum lactate buffer, (4) washing with water and ethanol, (5) lyophilization, and (6) extraction of glutenin with sodium dodecyl sulfate-β-mercaptoethanol-Tris borate buffer. Samples of gliadin and glutenin can then be analyzed by aluminum lactate and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Using this procedure, an extensive series of D-genome durum substitution and addition lines has been analyzed as part of a

study to determine the chromosomal control of gliadin and glutenin polypeptides in Langdon durum. One high-performance liquid chromatographic gel filtration method for protein extracts was tested with numerous wheat varieties as a possible technique useful for selection and identification, as well as for preparative purposes. Although class and varietal differences in proteins could be demonstrated, the column resolution and range were not optimal, suggesting that trials with other newer columns may be more successful.

- c. Specific Objective: To establish whether specific proteins are produced in larger amounts in newly developed high-protein wheats in order to better understand functional properties and genetic differences in these wheats.

Progress: Extraction and gel filtration of wheat proteins from a number of experimental wheats were carried out. Many of these new lines had high protein or high lysine content. The separated proteins are being further evaluated by electrophoresis.

- d. Specific Objecitive: Determine the effect of introgression of Tripsacum genes into corn on protein composition.

Progress: Genes from Tripsacum, a close relative of corn, were introduced into corn by crossing and selection at the University of Illinois. The grain of the progeny had higher protein content than that of the parent corn inbred; however, there was no major change in amino acid content. Tripsacum genes in corn may result in only subtle changes in zein components rather than changes in relative amounts of the different solubility classes of proteins. Methods were, therefore, developed to enable studies of differences in zein polypeptide compositions. Zein polypeptides were extracted and then separated on polyacrylamide gels by isoelectric focusing; the resultant stained patterns were analyzed by scanning densitometry, and the acquired data were evaluated with the aid of a computer. These techniques have been applied to analysis of zeins extracted from grains of various races of corn indigenous to different geographic areas of North and South America and were used to develop relationships between the races of corns based on similarities of electrofocusing patterns.

Publications:

BIETZ, J. A. Amino-Terminal Amino Acid Sequence of Hordein. Cereal Chem. 58 (in press).

BIETZ, J. A., AND F. R. HUEBNER. Structure of Glutenin: Achievements at the Northern Regional Research Center. Annales de Technologie Agricole. In press.

BIETZ, J. A., AND J. S. WALL. Identity of High Molecular Weight Gliadin and Ethanol-Soluble Glutenin Subunits of Wheat: Relation to Gluten Structure. *Cereal Chem.* 57 (1980):415-421.

BIETZ, J. A., AND V. G. RYADCHIKOV. Chairman Session Report, Workshop on the Physicochemical Properties of Wheat Gluten Proteins; Session V: Gluten. *Annales de Technologie Agricole.* In press.

ESEN, A., J. A. BIETZ, J. W. PAULIS, AND J. S. WALL. Fractionation of Alcohol-Soluble Reduced Corn Glutelins on Phosphocellulose and Partial Characterization of Two Proline-Rich Fractions. *Cereal Chem.* Submitted for publication.

HUEBNER, F. R., AND J. S. WALL. Wheat Glutenin: Effect of Dissociating Agents on Molecular Weight and Composition as Determined by Gel Filtration Chromatography. *J. Agric. Food Chem.* 28(2) (1980):433-438.

PAULIS, J. W. Recent Developments in Corn Protein Research. *J. Food Sci.* Submitted for publication.

WALL, J. S. Corn Processing. In *Encyclopedia of Science and Technology*, McGraw-Hill Co., New York, NY. In press.

Other Reports:

BIETZ, J. A. Genetic Interrelationships of Cereal Grains Revealed Through Prolamin Sequence Analysis. Presented at the AACC 65th Annual Meeting, Chicago, IL, September 21-25, 1980.

BIETZ, J. A. Maximizing Use of Normal- and High-Lysine Sorghums in Foods. Presented at the 2nd International Symposium on Sorghum and Millets for Human Foods, Vienna, Austria, May 1980.

BIETZ, J. A. Protein Sequence Analysis Aids in Understanding Quality and Origin of Cereal Grains. Presented at the Conference of General Collaborators from North Central Agricultural Experiment Stations at the Northern Regional Research Center, Peoria, IL, October 20-21, 1980.

BIETZ, J. A. Structure of Glutenins: Actual Achievement of the Peoria Group's Work. Presented at the Workshop on the Physicochemical Properties of Wheat Gluten Proteins, Nantes, France, April 28-30, 1980.

HUEBNER, J. R., AND J. S. WALL. Protein Composition of Protein Bodies from Immature Wheat Kernels Using Electron Microscopy. Presented at the 2nd Chemical Congress of the North American Continent, ACS, Las Vegas, NV, August 25-29, 1980.

PAULIS, J. W., D. FEY, W. F. KWOLEK, AND M. M. GOODMAN. A Grouping of Latin American Races of Maize by Zein Isoelectric Focusing Pattern. Presented at the 72nd Annual Meeting of American Society of Agronomy, Detroit, MI, November 30-December 5, 1980.

WALL J. S. Glutelin Proteins in Maize and Wheat: Chemistry, and Origins. Presented at U.S.-Australia Workshop on Biology of Seed Protein Synthesis and Deposition, Honolulu, HI, February 4-8, 1980.

WALL J. S. Progress Report on Cereal Protein Research at NRRC. Presented at the Annual Meeting of NC 151 Committee, Kansas City, MO, January 3-4, 1980.

WALL, J. S. Wheat Protein Research at NRRC. Presented at Meeting of the American Bakers Association Technical Liaison Committee with the USDA, Albany, CA, February 13-15, 1980.

WALL, J. S., AND F. R. HUEBNER. Adhesion and Cohesion. Presented in Symposium on Functional Properties Governing Roles of Protein in Food Applications, Agricultural and Food Chemistry Division of ACS, Houston, TX, March 24-25, 1980.

WALL, J. S., J. E. SANDERSON, G. L. DONALDSON, AND J. H. SLONEKER. Determination of Total Bound Inositol and Phytic Acid by Gas-Liquid Chromatography of Inositol Acetate. Presented before Agricultural and Food Chemistry Division of ACS, Houston, TX, March 24-25, 1980.

6. Epoxides from Lipid Hydroperoxides and Their Interactions in Cereal and Oilseed Foods (H. W. Gardner)

- a. Specific Objective: Study the pathways of fatty acid epoxide formation from fatty acid hydroperoxides and test both the epoxides and hydroperoxides for toxicity and/or mutagenicity by the Ames test.

Progress: Homolytic decomposition of hydroperoxides of polyunsaturated fatty acids often results in fatty epoxides, which are potentially toxic compounds. We have now assessed the mutagenic activity of the fatty epoxides and the parent hydroperoxides by the Ames test. The fatty epoxides were found to be not mutagenic; however, the parent hydroperoxide was toxic and weakly mutagenic to certain strains of Salmonella typhimurium used in the Ames test. Also, the formation of fatty epoxides was studied with regard to the nature of the metal catalyst used. Kinetically, it was found that inorganic iron was degrading fatty acid hydroperoxide to epoxides much like an enzyme. Two electrons were required for reduction of the iron catalyst for every mole of hydroperoxide degraded. This electron stoichiometry accounted for a second oxidation in the reaction. The optical activity of six different epoxide products was examined, and it was found that the optical

activity of the parent compound, 13-L(S)-hydroperoxyoctadecadienoic acid, was retained in all epoxide products, giving further proof that the epoxides were generated through rearrangement of an alkoxy radical intermediate.

Publications:

GARDNER, H. W., C. G. CRAWFORD, AND J. T. MACGREGOR. Fatty Epoxides Derived from Degradation of Linoleic Acid Hydroperoxides are not Mutagenic by Ames Test. Proceedings, National Cancer Institute Workshop on Lipid Oxidation, Vitamin E, Selenium and Carcinogenesis, Rockville, MD, September 15-16, 1980 (in press).

Other Reports:

GARDNER, H. W. Free Radical Reactions Induced by Lipid Peroxidation and Implications for Meat Quality and Safety. Seminar at Meat Science Research Laboratory, Agricultural Research, Beltsville, MD, September 18, 1980.

GARDNER, H. W., AND P. A. JURSINIC. Further Studies of a Model for Lipid Hydroperoxide Degradation: Characterization of New Products and the Non-participation of Superoxide Anion in the Formation of Secondary Oxidation Products. ISF/AOCS Meeting, New York, April 27-May 1, 1980.

GARDNER, H. W., C. G. CRAWFORD, AND J. T. MACGREGOR. Fatty Epoxides Derived from Degradation of Linoleic Acid Hydroperoxides are not Mutagenic by Ames Test. National Cancer Institute Workshop on Lipid Oxidation, Vitamin E, Selenium and Carcinogenesis, Rockville, MD, September 15-16, 1980.

7. Molecular Structure of Maillard-Type Browning Reaction Products (E. B. Bagley)

- a. Specific Objective: Conduct sugar-amine browning reactions under cereal food processing conditions of heat treatment to produce and isolate several fractions of the many soluble reaction products for compositional analysis, so that detailed knowledge will be obtained on the nonvolatile products of Maillard-type browning reactions in processed foods.

Progress: It is assumed that free amino acids as well as amino groups of peptides and proteins in bread act on the disaccharides lactose and maltose in Maillard-type browning reactions during baking to form maltol and isomaltol. Experimental work carried out in this period supports this view. O-Galactosoylisomaltol (OGI) was prepared and isolated from this reaction of lactose with secondary amino acids in pepecolinic, proline and sarcosine, though in low yield. It was found that ion exchange resin (H form)

column chromatography was effective in isolating the OGI at reaction yield levels of 7% or less. It was also discovered that OGI also resulted from the reaction of lactose with the primary amino acid, glycine, at less than 5% yield.

- b. Specific Objective: Isolate and determine the chemical structures of the most active antioxidants in fractions of sugar-amino reaction mixtures to protect unsaturated lipids from autoxidation and possibly to provide better food-grade antioxidants than those now in use.

Progress: The oxygen-uptake method (developed in 1979) for the measurement of antioxidant activity was modified to yield better reproducibility (S.D. 7%). Application of the technique to ten different model, amino acid-glucose, browning reactions that were divided into organic soluble (O.S.), nondialyzable (N.D.) and dialyzable (D.) fractions confirmed previously reported antioxidant activity in browning reactions. The current work pinpointed where this activity exists.

Relative to butylated hydroxytoluene (BHT, PF=1.0), equal weight quantities in safflower oil of the latter fraction (mostly glucose and amino acid) were inactive (PF <0.37). When the amount of D fraction was increased tenfold, serine, methionine, and alanine fractions had PF's at 0.46, a slight activity.

Although full analysis of the two other fractions is not complete, it was demonstrated that the O.S. fractions, except for glycine, isoleucine, and lysine, had mild activity; when the fraction amounts were increased tenfold, all ten fractions had excellent antioxidant activity (PF=0.75-3.17). The nondialyzable fractions at equal weight amounts, except for glycine, isoleucine, and tryptophan, were not active. But increasing the concentration to ten times the standard BHT produced high activity for all the polymers except for those of leucine, lysine, and alanine (PF 0.4).

- c. Specific Objective: Determine the decomposition products of D-fructose in browning reactions with a pure protein containing a high content of lysine, or with a known peptide containing one or more lysine residues, to define reaction products that do not arise by condensation of fructose with the epsilon-amino group of the lysine residue.

Progress: The initial plan to synthesize the lysine-fructose was based on a literature procedure. It was discovered that the literature was incorrect. The correct reaction pathway was worked out, and it was shown that the reaction group intended as the point of entry for the lysino group was destroyed in the process. Consequently, a new approach to the synthesis of the lysino fructose was conceived and is being investigated.

d. Specific Objective: To complete work on glycol configuration.

Progress: The series of reactions on methyl tri-O-methanesulfonyl-a-D-glucopyranoside with base was completed. This work relates to the reactivity of particular sites in the glucopyranosyl molecule as a guide to understanding of enzymatic action. After the initial displacement to give an epoxy methane sulfonyl derivative, four reactions were discovered and an order of preference in product determination was worked out.

Publications:

GOODWIN, J. C., J. E. HODGE, AND D. WEISLEDER. Preparation of Bicyclic Hexitol Anhydrides by Using Acidic Cation-Exchange Resin in a Binary Solvent. ^{13}C -N.m.r. Spectroscopy Confirms Configurational Inversion in Chloride Displacement of Methanesulfonate in Isomannide and Isosorbide Derivatives. *Carbohydr. Res.* 79 (1980):133-141.

GOODWIN, J. C., J. E. HODGE, E. C. NELSON, AND K. A. WARNER. Structure-Taste Relationships Among Cyclic Glycols, Levoglucosan, and Methyl Glycopyranosides. Submitted for publication in *J. Agric. Food Chem.* (1980).

GOODWIN, J. C. AND J. E. HODGE. Sweetness and Bitterness of Some Aliphatic α,ω -Glycol D-Glucopyranosides. Submitted for publication in *J. Agric. Food Chem.* (1980).

FLIPPIN-ANDERSON, J. L., AND F. D. MILLS. Molecular and Crystal Structure of a Diindenopyran Isolated from the Acid Hydrolysis of Amino-Hexose-Reductones. For publication in *Journal of Crystallography*. In preparation.

HODGE, J. E. Food and Feed Uses of Corn. Submitted for CRC Handbook Series in Agriculture, Processing and Utilization (1980).

MILLS, F. D. 3-Methyl-1H-Indenones: A One Step Conversion from 2,3-Dihydro-1H-Indenones with N-Bromosuccinimide. Accepted for publication in *J. Org. Chem.* (1980).

MILLS, F. D. Preparation and Catalytic Hydrogenation of 4,6-Dimethyl- and 4,7-Dimethyl-8-Methoxycoumarins. A 4-Methyldihydrocoumarin Synthesis. *J. Heterocyclic Chem.* 17 (1980):1597-1600.

MILLS, F. D., J. E. HODGE, AND L. J. TJARKS. Structure, Relative Conformation, and Synthesis of a Condensed Aromatic Antioxidant from the Acid Hydrolysis of Amino-Hexose-Reductones. Submitted for publication in *J. Org. Chem.*

MILLS, F. D., J. F. CAVINS, AND F. R. HUEBNER. The Source of Antioxidants Formed in Model Browning Reactions. For publication in JAOCs. In preparation.

SINCLAIR, H. B. Products from the Base Treatment of the Tri-O- and Tetra-O-Methanesulfonyl Esters of Methyl α -D-Glucopyranoside. Submitted for publication in J. Org. Chem. (1980).

SINCLAIR, H. B. Reactions of the Six Methyl Di-O-Methanesulfonyl α -D-Glucopyranosides with Base. For publication in J. Org. Chem. In press.

SINCLAIR, H. B. Action of Base on the Di-O-Methanesulfonyl Esters of Methyl α -D-Glucopyranoside: Displacement Order and End Products. J. Org. Chem. 44 (1979):3361-3368.

VAN CLEVE, J. Ethylidene Derivatives of D-Erythrose, II. Synthesis of 2',3'-O-Ethylidene- β -D-Erythofuranosyl 2,3-O-Ethylidene- β -D-Erythofuranoside. In preparation for Carbohydrate Research.

VAN CLEVE, J. Methyl 4,6-O-Benzylidene- α -D-Glucopyranoside. Submitted for publication in Methods in Carbohydrate Chemistry (1980).

VAN CLEVE, J. Synthesis of β -D-Erythofuranosyl β -D-Erythofuranoside. In preparation for Carbohydrate Research.

VAN CLEVE, J. The Allyl and Benzyl Glucosides of D-Erythrose. In preparation for Carbohydrate Research.

B. TECHNOLOGIES FOR INDUSTRIAL USES OF PLANT AND ANIMAL PRODUCTS

1. Enzymatic Conversion of Cellulose to Sugars for Alcohol Fermentations (H. L. Griffin)

a. Specific Objective: Purify and characterize the swelling factor (C_1 component) isolated from the cellulase complex.

Progress: Chromatographic techniques for the isolation of the C_1 fraction directly from cellulase preparations were abandoned. Laboratory-scale instrumentation processes small (10 ml) samples, and the isolated products are unstable. Two factors have increased the efficiency of the fractionation and the stability of the fractions: (1) during cellulolysis, the cofactors become associated with the sugar products and can be isolated in a stable mixture, and (2) an Amicon Hollow fiber ultrafiltration allows rapid separation of the mixture from about 1 liter of enzyme preparation. The sugars and cofactors that pass through an Amicon P2 hollow

fiber are free of enzyme activity, swell cellulose, and increase the solubilizing and saccharifying activity of a complete cellulase preparation. Little difference was found in the neutral carbohydrate composition of stalks and cobs from a variety of geographic and genetic sources.

Publication:

KRULL, L. H. AND G. E. INGLETT. Analysis of Neutral Carbohydrates in Agricultural Residues by Gas-liquid Chromatography. J. Agric. Food Chem. 28 (1980):917-919.

2. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

See Northern Agricultural Energy Center, A.4.

C. CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED

1. Effect of Environmental Contaminants on Cereal Foods and Feeds (W. J. Garcia)

- a. Specific Objective: Assess the effects of chronic levels of ambient ozone on field-grown corn plants with research emphasis on changes in composition of the edible grain.

Progress: General conclusions, derived from the completed study involving corn plants grown at ambient ozone levels ranging from 0.02 to 0.15 ppm O₃, were that stress effects due to ozone exposure were more readily manifested in the vegetative plant at lower levels of ozone than in the edible grain. Physical characteristics such as foliar injury and suppression of plant size and crop yield were easily detectable at lower ozone concentration levels, whereas corn kernel size and composition changes in the grain initially occurred at higher ambient ozone levels (0.15 ppm). Compositional changes in the grain recovered, from plants exposed to all levels of ozone studied, were minor for: protein, amino acids, fat, fiber, ash, starch, and amylose content of starch. Essentially, no difference in macromineral element (potassium, phosphorus, and magnesium) levels was found at all levels of ozone exposure; however, levels of certain trace elements (zinc, iron, copper, and cadmium) generally increased with an increase in ambient ozone concentration. By contrast, levels of manganese and chromium in the grain were essentially unchanged at different ozone concentrations. Response to the same levels of ambient ozone were different for three corn varieties.

Publications:

GARCIA, W. J., C. W. BLESSIN, G. E. INGLETT, W. F. KWOLEK, J. N. CARLISLE, L. N. HUGHES, AND J. F. MEISTER. Metal Accumulation and Crop Yield for a Variety of Edible Crops Grown in Diverse Soil Media Amended with Sewage Sludge. Submitted to Environ. Sci. Technol.

Other Reports:

GARCIA, W. J. Metal Accumulation and Crop Yield for a Variety of Crops Grown in Diverse Soil Media Amended with Sewage Sludge. Presented at the 14th Middle Atlantic Regional Meeting of the American Chemical Society, Valley Forge, PA, April 23-25, 1980.

GARCIA, W. J. Potentials of Sludge. Illinois Operator, September 1980 issue.

GARCIA, W. J. Translocation of Metals from Diverse Soils to Food Crops as Determined by Atomic Absorption. Presented at the 5th Annual Association of Official Analytical Chemists Spring Workshop, St. Louis, MO, April 7-10, 1980.

D. FOOD COMPOSITION AND IMPROVEMENT

1. Action of Human Digestive System Upon Cereal Grain Fiber Sources and Related Foods (F. R. Dintzis)

- a. Specific Objective: To examine factors influencing mineral binding properties of fiber sources such as wheat brans.

Progress: Studies using radioactive iron with four different wheat brans reveal that after 4-hour contact in vitro with solutions simulating human gastric conditions, both pericarp material and endosperm residues strongly bind ferric iron and, to a significantly smaller extent, ferrous iron. Zinc is not significantly bound by wheat bran at high acid conditions of pH ~1.5.

- b. Specific Objective: To cooperate with the SEA-AR Human Nutrition Research Laboratory in order to determine the effects of dietary fiber sources in human nutrition.

Progress: The total phosphorous and phytate phosphorus contents of some dietary fiber sources have been determined in order to see if a correlation exists between these variables and measured physiological effects. Dietary fiber composition of bran remnants retrieved from feces of human volunteers continues to be measured and evaluated.

Publications:

DINTZIS, F. R., AND C. HARRIS. Starch Determination in Some Dietary Fiber Sources. Manuscript being revised for resubmission to Cereal Chemistry.

DINTZIS, F. R. Dietary Fiber Analysis--Concepts and Problems. Manuscript in preparation for IFT Symposia, June 1981.

Other Reports:

DINTZIS, F. R., AND C. HARRIS. Starch Determination in Some Dietary Fiber Sources. Presented at the 65th Annual AACC Meeting, Chicago, IL, September 24, 1980.

SANDSTEAD, H., L. KLEVAY, D. MILNE, J. MAHALKO, F. DINTZIS, G. INGLETT, AND V. YOUNG. Effect of Dietary Protein and Fiber on Mineral Requirements of Man. Presented at the 65th Annual AACC Meeting, Chicago, IL, September 24, 1980.

FERMENTATION LABORATORY

C. W. Hesseltine, Chief

Research Leaders: R. W. Dstroy, M. D. Grove, T. G. Pridham,
O. L. Shotwell, and M. E. Slodki

A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Polysaccharides in Specific Associations of Nitrogen-Fixing Microbes with Plants (M. E. Slodki)

- a. Specific Objective: Continue cooperative studies with Charles F. Kettering Research Laboratory on structures of Rhizobium japonicum 138-type exopolysaccharides.

Progress: Capsular polysaccharides (CPS) isolated from logarithmic and stationary growth phases of R. japonicum 110 and 138 cultures were carboxyl-reduced by the ethylene oxide/NaBD₄ method and then pertrideuteriomethylated by the Hakomori procedure using CD₃I. Following hydrolysis and conversion to per-O-acetylaladononitriles, the methylated sugars were separated by g.l.c. and analyzed by both ion impact and chemical ionization (isobutane as reagent gas) mass spectrometry. Complete reduction of galacturonic acid residues now gave component molar ratios that accorded with analyses of the unmethylated polysaccharides. The CI mass spectra permitted direct estimation, as molecular ions, of the relative amounts of C-6 dideuterated and undeuterated components of the g.l.c. peak corresponding to the 2,4,6-tri-O-(trideuterio) methyl galactose derivative. In all preparations, the C-6 undeuterated component constituted approximately 10% of the peak area and is thought to reflect substitution of a portion of the (1→3)-linked D-galacturonic acid residues by D-galactose. According to the CI spectra, natural 4-O-methylation of D-galactosyl end groups increases from 40% at 4 days to 60% at 11 days in CPS 110; for CPS 138, the corresponding values were 76 and 66. The results of methylation structural analysis previously obtained for a reduced pentasaccharide derived from CPS 138 were confirmed on a new preparation.

- b. Specific Objective: Study changes, during stages of culture growth, in noncarbohydrate substituents of exopolysaccharides of fast-growing rhizobia.

Progress: If they are indeed determinants of nodulation specificity, then the well-documented, close similarity in compositional analyses of exopolysaccharides (EPS) from Rhizobium leguminosarum, R. phaseoli,

and R. trifolii must be explained. Based on experience with the EPS of Xanthomonas campestris, changes in noncarbohydrate substituents were examined as a function of culture age. (Transient binding of R. trifolii EPS with clover lectin as a function of culture age has been reported.) The pyruvic acetal contents of EPS from the above species as well as from R. meliloti increase with culture age. O-Acetyl content, however, increases only in the EPS of R. leguminosarum and R. trifolii, remains unchanged in R. phaseoli EPS and decreases in R. meliloti EPS. Variations in both substituents also were observed between strains of all four species and, in R. meliloti EPS, depended on whether glycerol or mannitol was the C source. Yields of R. meliloti EPS were significantly higher from glycerol, whereas the other species produced more EPS from mannitol. It was found that rhizobial culture pH can be controlled by varying the component levels of a dual mannitol-potassium gluconate carbon source.

- c. Specific Objective: Determine whether production of rhizobitoxine by certain strains of R. japonicum affects methylation of exopolysaccharides.

Progress: With respect to capsular (CPS) or extracellular (EPS) polysaccharides, there are two types of R. japonicum: one produces a CPS that contains D-galactose nonreducing end groups which are partially 4-O-methylated; the other type forms an EPS composed of L-rhamnosyl main chains to which are appended 4-O-methyl-D-glucuronic acid residues. Strains reported to either produce rhizobitoxine, an inhibitor of the biosynthesis of one of the precursors of the methyl donor methionine, or to induce chlorosis were examined with regard to the type of polysaccharide formed. It was found that all but one of the strains tested were of the type that form the rhamnose/methylglucuronic EPS. This type generally displays acetylene reduction activity; the other type is generally inactive or displays weak activity. Unmethylated glucuronic acid was not found in the EPS. The lone chlorosis-inducing strain that forms the 4-O-methyl galactose/galactose CPS, USDA 61, had these sugars in relatively low molar ratio to each other compared to other rhizobia that make a similar CPS; acetylene-reduction activity was also high.

- d. Specific Objective: Determine the factors involved in preferential nodulation of soybean varieties by strains of R. japonicum.

Progress: There is much evidence that exopolysaccharides of R. japonicum strains are involved in specific recognition between the microsymbionts and their legume hosts. On the part of the host, this recognition supposedly is mediated by a form of the seed lectin found in root hairs of Glycine max. Soybean lectins, however, have D-galactose specificity even though many strains of R. japonicum

form an exopolysaccharide consisting of L-rhamnose and 4-O-methyl-D-glucuronic acid and have no D-galactose residues. It has been reported that the Peking variety is nodulated preferentially by USDA strains 76-94 (rhamnose/methyl glucuronic polysaccharides), whereas the Beeson variety is preferentially nodulated by USDA strain 110 (galactose/4-O-methyl galactose polysaccharide). Lectins of D-galactose specificity were isolated from seeds of both varieties by affinity chromatography on immobilized N-acetyl-D-galactosamine followed by elution with D-galactose. Fluorescein isothiocyanate-labeled Beeson lectin bound to cells of R. japonicum 138 (polysaccharide similar to 110), but not to cells of strain 94. Beeson lectin was tested by immunodiffusion against Lipomyces starkeyi NRRL Y-1388 polysaccharides which have angle unit side chains of O-acetylated D-galactose. A precipitin band formed with the native, but not the deacetylated, polysaccharide. After lyophilization, the lectin also reacted with the deacetylated form. Evidently, physical treatment of soybean lectin can alter its carbohydrate binding specificity.

Publications:

SLODKI, M. E. Structural Aspects of Exocellular Yeast Polysaccharides. ACS Smposium Series 126. Fungal Polysaccharides, edited by P. A. Sandford and K. Matsuda, American Chemical Society, Washington, D.C., 1980, pp. 183-196.

JACKSON, L. K., M. E. SLODKI, M. C. CADMUS, K. A. BURTON, AND R. D. PLATTNER. 3-O-Methyl-L-Rhamnose from a Rhizobium Capsular Polysaccharide. Carbohydr. Res. 82 (1980):154-157.

CHERNIAK, R., E. REISS, M. E. SLODKI, R. D. PLATTNER, AND S. O. BLUMER. Structure and Antigenic Activity of the Capsular Polysaccharide of Cryptococcus neoformans Serotype A. Mol. Immunol. 17 (1980):1025-1032.

SLODKI, M. E. Extracellular Microbial Polysaccharides. Encyclopedia of Chemical Technology, Third Ed., edited by M. Grayson and D. Eckroth, Wiley Interscience, New York, NY, 1981, accepted for publication.

Other Reports:

CADMUS, M. C., K. A. BURTON, L. K. JACKSON, AND M. E. SLODKI. Compositional Changes in Rhizobial Polysaccharide During Growth. Seminar on Microbial Polysaccharides of Industrial Significance. 80th Annual Meeting American Society for Microbiology, Miami Beach, Florida, May 15, 1980.

2. Physiology of Nitrogen-Fixing Blue-Green Algal and Rhizobial Symbioses with Plants (J. W. Newton)

- a. Specific Objective: To study any unique proteins elaborated during development of the Azolla-blue-green algal nitrogen-fixing symbioses.

Progress: Even though improvements were made in the procedure used for high resolution, radioautographic, two-dimensional electrophoresis, proteins from crude plant preparations were less readily resolved than those from algal extracts. This problem made comparisons between preparations ambiguous. Consequently, comparative work was undertaken using algal bundle preparations isolated from plants, the isolated free-living blue-green algae and crude plant preparations. The algal bundle preparations derived from plants grown in the presence of $^{14}\text{CO}_2$ did show a distinctive protein that was not present in either the free-living algae or whole plants.

- b. Specific Objective: Isolation of blue-green algae from worldwide collection of Azolla species.

Progress: In addition to A. caroliniana, we have now succeeded in obtaining A. mexicana and A. pinata plants that apparently are free of bacterial and fungal contamination. Other Azolla species are being repeatedly surface-sterilized in attempts to obtain additional aseptic species. New free-living algal strains have not yet been obtained from these plants by use of techniques that were effective with A. caroliniana.

- c. Specific Objective: Study the life cycle of Azolla mexicana to determine the fate of the algal symbiont.

Progress: Plants were successfully obtained from crude spore preparations of A. mexicana and work is continuing on separation and isolation of stages in the life cycle. Successful germination of spores indicates that the preparations in hand are capable of completing the life cycle and could enable separation and isolation of the stages for experiments on reintroducing the algal symbiont--provided one of the stages can be rid of the symbiont. Recently, a plant tissue culture has been obtained under aseptic conditions from minced A. caroliniana.

- d. Specific Objective: Rhizobial strains with different phenotypes will be examined for their NH_4^+ metabolism and subsequently transformed to spheroplastic cells.

Progress: Two acetylene-reducing (AR^+) strains (32H1, L-259) and an AR- strain (L-246) were examined for glutamine synthetase (GS) activity under the microaerobic condition that elicits AR^+ activity. During the period of increasing AR^+ activity, the AR strains

displayed decreases in glutamyl transferase and forward GS activities and in extractable protein per unit wet cells. In contrast, the AR strain yielded extracts displaying no changes in transferase and forward GS activities even though there was also a decrease in extractable protein. This finding accords with the role of rhizobia in the export of fixed N to the plant.

The second phase of the work (spheroplasts deficient in cell-wall components) was facilitated by the finding that pleiomorphic (cell-wall defective) cells might be active in reduction of acetylene. On the assumption that these cells might also be capable of mating, a cross was made on glutamate-containing (GMG) agar between strains L-259 (phenotype AR⁻, GMG⁺, Km^s, Nm^r, Pn^s, Pm^r, Str^s, Tet^r) and L-281 (phenotype AR⁺, GMG⁺, Km^t, Nm^t, Pn^s, Pm^t, Str^s, Tet^t) to yield a hybrid of phenotype (AR⁺, GMG⁺, Km^r) at apparent low frequency. Spheroplastic cells of uniform population can be made by combined treatment with lysozyme and polymyxin B, but their viability and mating capability have not been tested. Cell fusion experiments employing pleiomorphs and spheroplasts might reveal whether genes for nitrogen fixation can be transferred at a high frequency between various cell-wall defective rhizobia.

- e. Specific Objective: Study conditions leading to pleiomorphism and free-living reduction of acetylene (AR⁺) by strains of Rhizobium japonicum and the cowpea-type rhizobia.

Progress: When grown on a glutamate-containing agar medium (GMG), a majority of R japonicum strains reduced acetylene (36 of 44 are AR⁺). A study of three selected AR⁺ strains revealed that (1) the agar surface cultures gave more consistent acetylene reductions than did liquid cultures, (2) glutamate was the preferred N source for growth leading to AR activity, and (3) a combination with gluconate-mannitol C sources yielded optimal AR activity. The AR⁺ test strains were characterized further by their glutamate synthetase activity, antibiotic sensitivity, colony type, cellular morphology, and differential growth on various N sources. It was concluded that glutamate stimulated aerobic growth leading to uniform glutamine synthetase activity, pinpoint colonies and pleiomorphism. Relating pleiomorphic cells to AR⁺ activity could resolve the question of whether pleiomorphic or rod-shaped forms in the mixed population fix N₂, provided the cell types can be separated.

Publications:

KANESHIRO, T., J. W. NEWTON, E. SELKE, AND M. E. SLODKI. Dinitrogen (¹⁵N₂) Fixation and Acetylene Reduction in Free-Living Strains of Rhizobium. Curr. Microbiol. 3 (1980):279-281.

NEWTON, J. W., AND E. SELKE. Assimilation of Ammonia by the Azolla-Anabaena Symbiosis. J. Plant Nutr. 3(3) (1981).

KANESHIRO, T. AND M. E. SLODKI. Glutamate as a Differential Nitrogen Source for Acetylene Reducing Rhizobium Cultures. Plant Physiol. (Suppl.) 65 (1980):110.

Other Reports:

NEWTON, J. Blue-Green Algae Associated with the Azolla Symbiosis. Seminar. Department of Agronomy and Soil Science, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. February 1980.

3. Nitrogen Contribution of Azolla spp. in Aquatic Farming Systems
(Cooperative Agreement - University of Hawaii)

- a. Specific Objective: Development of Azolla management methods for wetland agricultural systems.

Progress: Agronomic studies on use of Azolla in rice cultivation in the People's Republic of China have now been completed. Six Azolla varieties were studied in a total of 108 rice plots of 26 square meters each. Rice yields varied from 4.5 to 6.1 tons/ha; highest yields were obtained when Azolla was used as a prior green manure followed by introduction at seedling transplant. This combined procedure introduced a high of 220 kg. N/ha; the lowest N content, 40 kg. N/ha, was obtained when Azolla was used as a top dressing only. Details will be presented at the NIFTAL/CIAT Nitrogen Fixation Workshop in Colombia in February 1981.

- b. Specific Objective: Isolation and characterization of blue-green algae associated with Azolla.

Progress: Using media selective for blue-green algae, 15 Anabaena-like algal strains were isolated from several different Hawaiian taro patches. About one-third of these strains have been purified. In addition, some new algal strains have been isolated from a rice paddy soil, which contains Azolla, that was collected in the People's Republic of China.

Publications:

LUMPKIN, T. AND D. L. PLUCKNETT. Azolla: Botany, Physiology and Use as a Green Manure. Economic Bot. 34 (1980):111-153.

B. BIOLOGICAL AGENTS FOR PEST CONTROL

1. Insecticidal Preparations of Bacillus thuringiensis and Other Microbial Insect Pathogens (D. E. Johnson)

- a. Specific Objective: Characterize sporulation mutant strains of Bacillus thuringiensis according to nutritional requirements and potential enzyme deficiencies.

Progress: Asporogenous mutants of B. thuringiensis that had been grown in either a complex medium or a nutritionally defined medium were characterized according to protease production. The synthesis of exoproteases in bacilli may be obligatorily linked to sporulation; that is, organisms which lack the enzyme(s) also fail to sporulate. In this case, however, all the asporogenous mutants examined retained the ability to produce extracellular protease at levels closely approximating the wild type. Since protease synthesis occurs at the onset of sporulation (Stage 0) in normal cells, the sporulation block in the mutants under examination must occur in some later event. Indeed, evidence of blockage at Stage 2 has been confirmed by electron microscopy.

- b. Specific Objective: Determine toxicity of crystals and activated toxin from B. thuringiensis against second instar larvae of the European corn borer.

Progress: Spore-free mutants of B. thuringiensis, which still form typical bipyramidal-shaped inclusion bodies, were assayed for toxicity toward larvae of the European corn borer (Ostrinia nubilalis). Probit analysis of the dose-mortality data indicated no statistically significant difference between the LD₅₀ of the crystals from the spore-free mutants as compared to the wild type (B. thuringiensis subsp. kurstaki HD-1) crystals (3.1 to 5.1 µg toxin protein/g diet). The toxicity of delta-endotoxin digested with α-chymotrypsinogen compared favorably (LD₅₀ ranged from 3.3 to 9.8 µg toxin protein/g diet) with the entomocidal activity of intact crystals, whether from the spore-free mutants or the wild type. Regression lines drawn from the mortality data were statistically parallel, indicating insignificant variation between HD-1 crystal potency and the potency of mutant spore-free crystals and/or enzyme-digested delta-endotoxin preparations. Surviving larvae exhibited significant weight reduction, reaching as much as 95% at LD₅₀ dosage levels.

- c. Specific Objective: Attempt to establish an insect tissue cell line from larvae of European corn borer for eventual use as an in vitro bioassay system.

Progress: Initial attempts were unsuccessful. Eggs of European corn borer are packaged within a gelatinous mass, making it difficult to surface sterilize a collection of eggs effectively. In addition, if one waits until the blackhead stage, the eggs tend to disperse and float more readily, making the preparation more difficult to handle. Several attempts to establish a continuous cell line all resulted in contamination.

Publications:

JOHNSON, D. E., D. M. NIEZGODSKI, and G. M. TWADDLE. Parasporal Crystals Produced by Oligosporogenous Mutants of Bacillus thuringiensis (Spo CR). Can. J. Microbiol. 26:486-491. 1980.

Other Reports:

JOHNSON, D. E. Toxicity of Bacillus thuringiensis Entomocidal Protein Toward Cultured Insect Tissue. Presented at American Society for Microbiology meeting, Miami Beach, Florida, May 11-16, 1980.

JOHNSON, D. E. The Development of Bacillus thuringiensis Spo⁻ Cr⁺ Mutants and Measurement of Their Entomocidal Toxicity. Presented at the SEA-FS Insect Pathology Workshop, New Haven, Connecticut, October 7-9, 1980.

JOHNSON, D. E. Ultrastructure and Entomocidal Activity of Bacillus thuringiensis Spo⁻ Cr⁺ Mutants. Presented at Eighth International Spores Conference, Woods Hole, Massachusetts, October 9-12, 1980.

C. TECHNOLOGIES FOR FOOD AND FEED
USES OF FIELD CROPS

1. Rapid Characterization of Yeasts Through Genetic and DNA/DNA Hybridization and Computer Analysis (C. P. Kurtzman)

- a. Specific Objective: Determine the extent of genetic and DNA homology of progeny from crosses of yeasts having low DNA complementarity.

Progress: DNA from both homothallic and heterothallic yeasts exhibiting limited ability to hybridize has been isolated, purified, and partially characterized.

Publications:

FELL, J. W., STATZELL-TALLMAN, A., AND KURTZMAN, C. P. Sterigmatomyces Fell. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P., M. J. SMILEY, C. J. JOHNSON, L. J. WICKERHAM, AND FUSON, G. B. Two New and Closely Related Heterothallic Species, Pichia amylophila and P. mississippiensis: Characterization by Hybridization and Deoxyribonucleic Acid Reassociation. Int. J. Sys. Bacteriol. 30 (1980):208-216.

KURTZMAN, C. P., M. J. SMILEY, AND C. J. JOHNSON. Emendation of the Genus Issatchenkovia Kudriavzev and Comparison of Species by Deoxyribonucleic Acid Reassociation, Mating Reaction and Ascospore Ultrastructure. *Int. J. Sys. Bacteriol.* 30 (1980):503-513.

KURTZMAN, C. P. Preservation of Fungi by Lyophilization and Liquid Nitrogen Freezing. In The Preservation of Microorganisms by Freezing and Freeze Drying, Society for Industrial Microbiology, Arlington, Virginia. 1980.

KURTZMAN, C. P. A Guide to Identifying and Classifying Yeasts (Book Review). J. A. Barnett, R. W. Payne, and D. Yarrow. Cambridge University Press, Cambridge, England, 1979, 315 pp. *Intern. J. System. Bacteriol.* 30 (1980):601.

KURTZMAN, C. P. Hemiascomycetes. In Taxonomy and Classification of Living Organisms, Edited by S. P. Parker, McGraw-Hill Book Co., New York, accepted.

KURTZMAN, C. P. Citeromyces Santa Maria. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P. Pachysolen Boidin et Adzet. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P. Issatchenkovia Kudriavzev. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P. Hansenula H. et P. Sydow. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P. Ascospores. In Yeast Cell Envelopes--Biochemistry, Biophysics and Ultrastructure, Edited by W. N. Arnold, CRC Press, Boca Raton, Florida, accepted.

PHAFF, H. J., AND C. P. KURTZMAN. Lipomyces Lodder et Kreger-van Rij. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, accepted.

Other Reports:

KURTZMAN, C. P., M. J. SMILEY, C. J. JOHNSON, L. J. WICKERHAM, AND G. B. FUSON. Two New and Closely Related Heterothallic Species of Pichia: Characterization by Hybridization and DNA Reassociation. Presented at the American Society for Microbiology meeting, Miami Beach, Florida, May 11-16, 1980.

KURTZMAN, C. P., M. J. SMILEY, C. J. JOHNSON, AND M. J. HOFFMAN. Deoxyribonucleic Acid Relatedness Among Species of Sterigmatomyces. Presented at Vth International Symposium on Yeasts, London, Ontario, Canada, July 20-25, 1980.

KURTZMAN, C. P. Preservation of Fungi by Lyophilization and Liquid Nitrogen Freezing. Presented at Society for Industrial Microbiology meeting, Flagstaff, Arizona, August 9-15, 1980.

2. Germ Plasm Bank of Microorganisms for Research on Plant Residue Utilization (T. G. Pridham)

- a. Specific Objective: Continue operation of the Agricultural Research Culture Collection (NRRL) including original and supportive research.

Progress: Bacteriology staff members of the Agricultural Research Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1981, the Collection maintained 72,483 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1980, the Collection distributed 3,348 strains, of which 2,271 were provided to investigators in the United States and 1,077 were sent abroad. Of 192 strains deposited in the patent collection, 130 were from foreign sources; 225 patent strains were distributed to United States researchers, and 193 to foreign. Three hundred eighty-five strains of bacteria and actinomycetes were identified to species for interested parties.

A review of the literature on nonlegume nitrogen fixation with special emphasis on microbiological aspects was continued. Studies of one representative strain of the actinomycete responsible for symbiotic dinitrogen fixation with Comptonia peregrina (Sweet Fern) were continued in an effort to determine conditions for maximal biomass production in vitro. Despite use of many different nutrient materials, growth still is very slow and sparse. It was concluded that precise determination of the gaseous environment necessary for maximal growth of the organism is necessary before any substantive results can be obtained with the variety of physiological tests available for characterization.

Steps were taken to obtain recognition of the Agricultural Research Culture Collection (NRRL) as an international depositary authority in connection with the World Intellectual Property Organization (WIPO). That recognition will become official in 1981 making the Collection one of two in the United States and one of twelve internationally recognized ones suitable for deposition of patent strains in accordance with the rules and regulations of the Swedish Patent Office, the European Patent Organization, and the World Intellectual Property Organization.

During 1980, another important collection of microorganisms, the original Waksman collection of actinomycetes, was rescued to guarantee its continued maintenance. The collection comprises some 630 strains accumulated by Nobel Laureate Dr. S. A. Waksman over the years since 1916.

- b. Specific Objective: Continue DNA homology studies of agriculturally significant bacteria.

A low range of DNA reassociation levels (5-33%) was observed between the new species Lactobacillus amylophilus and those species (L. bulgaricus, L. casei, L. coryniformis, L. delbrueckii, L. helveticus, L. salivarius, and L. xylosus) with which it shared one or more phenotypic characteristics such as the production of L-(+)-lactic acid, a common sugar fermentation pattern, or guanine plus cytosine (G + C) content of 45 mol. %. The lack of DNA relatedness was also noted between L. amylophilus and other homo-fermentative lactobacilli. DNA reassociation of 90-98% was measured among the L. amylophilus strains.

Studies of 20 strains showed that bacteria identified as Bacillus circulans can be separated into three groups on the basis of G + C contents of the DNA. One group has a G + C content of about 38 mol. %, the second about 50 mol. %, and the third about 53 mol. %.

- c. Specific Objective: Continue study of the relationship between molds from a seed-caching economy (ancient storage methods) and those from present-day cereal agroecosystems.

Progress: Contrasted with the great majority of Aspergillus oryzae and A. sojae, these A. flavus strains have become rodent domesticated and morphologically distinct.

Publications:

KURTZMAN, C. P. Preservation of Fungi by Lyophilization and Liquid Nitrogen Freezing. In The Preservation of Microorganisms by Freezing and Freeze Drying, Society for Industrial Microbiology, Arlington, VA, pages 4-12. 1980.

NAKAMURA, L. K. Lactobacillus amylovora, a New Starch-Hydrolyzing Species from Cattle Waste-Corn Fermentations. Int. J. Syst. Bacteriol. 1980. Accepted for publication.

PRIDHAM, T. G. Actinomycetales and Related Microorganisms. In Taxonomy and Classification of Living Organisms, McGraw-Hill Book Company two-volume reference work. Accepted for publication.

PRIDHAM, T. G., AND A. J. LYONS. Methodologies for Actinomycetales with Special Reference to Streptomycetes and Streptoverticillia. In Actinomycete Taxonomy, Edited by A. Dietz and D. W. Thayer, Soc. Ind. Microbiol. Special Publ. No. 6, 152-224. 1980.

ROGOSA, M., R. H. LAWSON, AND M. I. KRICHEVSKY (Eds.). National Work Conference on Microbial Collections of Major Importance to Agriculture. Phytopathology (in press as Special Publication). (T. G. Pridham and C. P. Kurtzman contributed inter alia to preparation of this publication.)

WALLEN, L. L., A. J. LYONS, AND T. G. PRIDHAM. Antimicrobial Activity of Patulin Derivatives: A Preliminary Report. J. Antibiot. 33 (1980):767-769.

WICKLOW, D. T., R. W. DETROY, AND S. ADAMS. Differential Modification of the Lignin and Cellulose Components in Wheat Straw by Fungal Colonists of Ruminant Dung: Ecological Implications. Mycologia 72 (1980):1065-1076.

WICKLOW, D. T., R. W. DETROY, AND B. A. JESSE. Decomposition of Lignocellulose by Cyathus stercoreus (Schw.) deToni NRRL 6473, A "White-Rot" Fungus from Cattle Dung. Appl. Environ. Microbial. 40:(1980):169-170.

Other Reports:

KURTZMAN, C. P. Preservation of Fungi by Lyophilization and Liquid Nitrogen Freezing. Presented at Society for Industrial Microbiology meeting, Flagstaff, Arizona, August 9-15, 1980.

PRIDHAM, T. G. Let's Talk it Out. Radio Talk Show on recombinant DNA strains of microorganisms, Radio Station WPEO, Peoria, Illinois, January 4, 1980.

PRIDHAM, T. G., Radio Interview on Culture Collection and recombinant DNA strains, Radio Station KMOX, St. Louis, Missouri, April 8, 1980.

3. Effect of Immobilization Procedure and Carrier on Enzymes That Hydrolyze Cereal Food Polymers (K. L. Smiley)

a. Specific Objective: Compare activity of immobilized versus soluble glucanases.

Progress: Laminarin solution was recycled through a column of Rhizopus oryzae QM-1032 beta 1,3-glucanase immobilized on charcoal, and product formation was studied by gel permeation chromatography. The results indicate formation of numerous oligosaccharides of molecular weight (MW) intermediate between those of laminarin

(4400) and glucose (180). Even though laminarin concentration decreases with time, its apparent molecular weight remains constant. This anomaly is not observed when laminarin is digested by the soluble form of the glucanase whereby the molecular weight of the laminarin decreases with time. The most probable explanation is that the recycling mode of the immobilized enzyme reactor allows unreacted laminarin to reside for varying lengths of time in the reservoir so that there is always some measurable, unreacted laminarin. Undoubtedly, a more reactive immobilized enzyme would more nearly resemble soluble enzyme. When lichenan was used as the substrate for immobilized QM-1032 β -glucanase, very little enzymic action was noted. The apparent MW of lichenan (Waters E-linear column) is greater than 2,000,000. This value is much larger than that of the soluble laminarin used previously with the immobilized enzyme. Apparently the larger β -glucans are sterically hindered from reacting with the solid state enzyme. Based on this observation, it seems unlikely that immobilized β -1,3-glucanases will be of any value for selective degradation of high molecular weight β -1,3-glucan polymers.

The β -glucanase complex produced by Cladosporium resinae was fractionated on a sephadex column into several components that degrade laminarin. Likewise, R. oryzae QM-1032 β -1,3-glucanase was isolated as a single component. The various components were tested for their ability to degrade partially oxidized laminarin. If the enzyme is of the exo acting type, an oxidized nonreducing terminal sugar component of the laminarin will block the enzymic reaction. Because their action is largely on internal bonds, endo-type enzymes will not be affected greatly by the oxidized terminal glucosyl groups of the glucan. The ratio of true glucose to reducing sugar is indicative of the type of enzymic action. The amount of reducing sugar formed from unaltered laminarin compared to that formed from partially oxidized laminarin will also indicate whether or not the enzyme is of the exo or endo type. Results show that C. resinae produces both endo and exo β -1,3-glucanases. The exo types give a 1:1 ratio of true glucose to reducing sugar value on laminarin and partially oxidized laminarin, but the amount of glucose released from the oxidized form is only about 0.1 to 0.25 that from unoxidized polymer. With the endo-type enzyme, the amount of reducing sugar formed is only slightly less from oxidized laminarin than from unoxidized; however, the ratio of true glucose to reducing sugar is 0.25 to 0.3 on either form of the polymer. QM-1032 also shows typical endo-type action.

- b. Specific Objective: Continue the search for unique endo- and exoglucanases.

Progress: Strains of Oerskovia xanthineolytica are known to produce an enzyme that will lyse live Saccharomyces cerevisiae cells. Yeast cell walls are known to contain a glucan that will cause

certain plants to elicit a phytoalexin useful in preventing fungal infection. It is of interest, therefore, to see if the Oerskovia yeast cell wall lysing enzyme would be of use in preparing cell wall fragments with elicitor properties. A strain of O. xanthineolytica carried in the NRRL Culture Collection as Arthrobacter luteus B-4567 was chosen for further study. In order to properly assess enzyme production-parameters, it was necessary to develop a lytic assay that was reasonably rapid and facile. A procedure was developed whereby the course of cell lysis was followed in a thermostated spectrophotometer cell as loss of turbidity.

Beta-1,3-glucan, necessary for induction of the lytic enzyme, was prepared from commercial active dry yeast. The optimum conditions for enzyme production were 0.3% yeast glucan in a salts medium incubated for 1 day at 30°C. Higher glucan concentrations repressed lytic enzyme formation completely. Also, longer incubation times resulted in loss of enzyme. Activated yeast can serve as an inducer, but lytic enzyme yields were only one-third to one-half that obtained by using the isolated glucan.

Culture filtrates of B-4567 show only minimal activity against laminarin. There is some correlation between lytic activity and laminarinase activity even though filtrates with no lytic activity had some laminarinase present. It is not clear at this point whether cell lysis and laminarin degradation are due to one or two enzymes.

Work is in progress on characterizing the products resulting from action of the B-4567 lytic enzyme on S. cerevisiae cell walls. A 50% methanol-insoluble polymer was formed that subsequently gave water-soluble and water-insoluble fractions. The methanol-insoluble/water-soluble fraction consists of two polymers. One is quite large having a MW of approximately 2×10^6 . The other polymer has an apparent MW of about 3×10^4 and accounts for about one-third of the carbohydrate present. The mixture was separated on a Sepharose 4B column. Upon acid hydrolysis, the higher MW polymer yielded only mannose. Also, when the high MW polymer was tested as a substrate for β -1,3-glucanase from both C. resinae and R. oryzae, no hydrolysis was evident, showing that the polymer is something other than a β -1,3-glucan. Work is still in progress on the remaining fractions.

4. Characterization and Classification of Mucorales from Cereal Grains and Their Raw Products (C. W. Hesseltine)

- a. Specific Objective: Continue collecting and studying isolates of Rhizopus and related genera from a wide variety of sources, especially cereal grains.

Progress: A total of 107 new strains of Mucorales representing 9, possibly 10, genera were added to the collection for classification and further detailed study. They were isolated from such varied sources as air spora, Artocarpus leaves, cotton fiber, Ephedra viridis seeds, animal feed, various insects, koji, patients, Indonesian ragi, soy flour, wheat straw, native and cultivated sunflower seeds and rotting heads, tempeh, tobacco leaves, and wood fibers.

- b. Specific Objective: Determine variation in size of morphological characteristics for type and authentic strains of Rhizopus species due to various growth parameters.

Progress: Size variation of morphological features due to media composition and temperature was found to be most apparent in species of the Rhizopus arrhizus-R. oryzae complex. An R. delemar strain was selected for intensive study because it appeared to be one of the more variable strains and because it has been used as an amylo mold in the industrial production of alcohol. Size measurements of sporangiospores were made and subjected to analysis of variance. The data support the notion that both media composition and temperature of incubation have a significant effect of sporangiospore size but that an incubation period of 5 to 14 days does not. An observed difference of mean length up to 4 μm due to difference in media offers a plausible explanation for differences in sporangiospore size for R. delemar and its varieties as reported by different workers, some of whom did not take into account variability due to media and temperature.

- c. Specific Objective: Continue classifying to species strains of Rhizopus sp. in the Collection.

Progress: One-hundred fifty-six isolates of Rhizopus were grown on appropriate media at two different temperatures and were examined. They were identified to species or tentatively placed near a current species concept. A total of 42 strains could not be readily assigned species names and require further detailed comparative study. Most of the questionably assignable strains are in the R. arrhizus and R. chinensis complexes.

- d. Specific Objective: Investigate the Mucoraceous molds in ragi.

Progress: Seven ragi (Oriental food starter) samples obtained from Indonesia were investigated; in each, Amylomyces was present, as well as large numbers of Saccharomycopsis fibuligera and a lactic bacterium. Studies indicate that these are the dominant organisms in each sample and that Rhizopus, formerly considered to be an essential component of the starter, is not required. The counts of these three components showed 10^2 - 10^4 for Amylomyces, 10^3 - 10^6 for S. fibuligera, and 10^5 - 10^6 for the lactic acid bacteria.

Publications:

ELLIS, J. J. The Effect of Medium, Temperature, and Age on Rhizopus delemar Sporangiospore Size. *Mycologia*. Accepted.

Other Reports:

ELLIS, J. J. Rhizopus oligosporus. Presented at Tempeh Workshop for Soycrafters Association of America, NRRC, Peoria, IL, July 11, 1980.

5. Fermentative Utilization of Cane Sugar Baggasses (P.L. 480 Grant - National Research Center, Cairo)

- a. Specific Objective: Utilization of cane sugar bagasses.

Progress: Selected microbial isolates (47) were investigated for the effect of the crude bagasse concentration as the sole carbon source in the culture medium on the production of SCP, extracellular cellulases, and hemicellulases. In most cases, SCP yield reached a maximal on using a bagasse concentration of 34.7 g/l.

No consistent relationship existed between the bagasse concentration in the culture medium and the production of cellulases and hemicellulases. The fungal isolates were the most potent and produced active extracellular CM-cellulases and hemicellulases. The bacterial isolates produced weak or no extracellular CM-cellulase and hemicellulase activities.

The effect of nitrogen source and level was tested by using NaNO₃ (fungal and Streptomyces isolates), yeast extract (YE), casein hydrolysate (CH) (bacterial isolates), triammonium phosphate (TAP), and corn-steep protein precipitate (CSPP) in the culture media. In these studies, 18 microbial isolates were investigated.

The biodegradation, by the selected fungal isolated Trichoderma viride 253, of cellulose and lignin in untreated (crude) and five different alkali-treated bagasse samples was investigated at different incubation periods. In most cases, decomposition of cellulose in bagasse at 7 days incubation contributed about 75% of that at 21 days incubation. In general, biodegradation of cellulose in the alkali-treated bagasse samples reached threefold that in the untreated sample. After 7 days and up to 21 days incubation, the rate of cellulose biodegradation in the alkali-treated bagasse samples was slow and depended on the biodegraded lignin.

The results also indicated that the SCP yield and cellulase and hemicellulase activities produced from NaOH-treated bagasse by T. viride 253 after 6 days incubation were similar to those found for

the untreated bagasse after 14 days incubation. On using NaOH-treated bagasse, the hemicellulase activity was maximal at the early stages of incubation and dropped thereafter.

6. Thermophilic Microbial Conversion of Cellulosic Materials to Animal Feed (P.L. 480 Grant - Academy of Agriculture, Poznan)

- a. Specific Objective: Isolation of thermophilic cellulolytic microorganisms and elaboration of simple technological methods for biosynthesis of single-cell protein.

Progress: (1) Thermophilic cellulolytic microorganisms were isolated and selected from natural substrates at temperatures above 45°C. (2) Several bacteria/fungi capable of high protein synthesis at thermophilic levels were classified. (3) High-lignin-degrading microbes isolated from natural substrates were characterized. (4) Isolation of high-cellulose producing microbes were isolated from highly digestible wheat straw residues.

D. TECHNOLOGIES FOR FOOD AND FEED
USES FOR ANIMAL PRODUCTS

1. Conversion of Feedlot Wastes into Feed Supplements by Fermentation with Grain (G. R. Hrubant)

- a. Specific Objective: Study continuous anaerobic fermentation of fresh swine waste combined with corn.

Progress: A laboratory silo was designed and constructed using a cylinder, top, and bottom composed of lucite plastic with capacity for silage of 14.2 X 109 cm. The lids were held in place by stepped construction and four steel rods with thumbscrews. Air tightness was obtained by rubber "O" ring fabrication. The vessel was fitted with five short tubes, each to accept number 10 rubber stoppers and symmetrically placed about the cylinder to serve as sampling ports under anaerobic conditions. The exit of silage liquid was provided by a perforated lucite disc which was hollowed on its lower surface as was the upper portion of the bottom lid to form a lens-shaped cavity. A center hole in the bottom lid connected to a horizontal shaft and terminated in a tube and valve to release excess fluid. Continuous silage compaction to stimulate farm silos was achieved by an air cylinder attached to a platform held on the lid of the silo. The cylinder shaft passed through a gland in the lid fitted with a rubber "O" ring and screwed into a steel plate to press downward on silage. A cylinder air pressure of 200 psi (14.06 kg/cm²) was used. The silo held an air pressure of 3 psi (0.2 kg/cm²) without leaks over periods of 2 weeks. The laboratory was modified to be able to isolate and identify anaerobic bacteria by use of the Hungate Method. This is a roll-tube technique and required installation of apparatus to handle tubes under anaerobic conditions,

congeal inoculated agar in smooth, thin layers on the walls of tubes, and provide oxygen-free gas by means of scrubbers and gas proportioning flow rate tubes. Selective media for certain groups of anaerobes were designed, and prereduced and anaerobically sterilized substrates were prepared. The laboratory silo was used to ferment swine waste and corn. Internal gas pressures did not exceed 3 psi (0.2 kg/cm²), and the heat of fermentation raised internal temperatures less than 0.6°C above ambient levels of 27.9°C. No variation of internal silo temperatures was found between the sites at five sampling ports. A lactic acid fermentation occurred with fetid odors changing to silagelike in 24 hours, and preservation was demonstrated of a moist feed ingredient (50% moisture) without the energy expenditure of drying.

- b. Specific Objective: Continue demonstration of pathogen-free capability of the continuous fermentation of corn with cattle waste by adding large doses of Mycobacterium paratuberculosis and bacterial viruses to the fermentor.

Progress: In the continuous fermentation of corn with feedlot waste liquid (FLWL), tests were repeated and show that: (1) M. paratuberculosis was killed within 10 hours; and viable cells--4% w/w (wet) of the first chamber content--did not pass into the second chamber. Cell numbers doubled in 10 days in the control (autoclaved FLWL:water = 1:3). (2) Five bacterial virus populations were substantially reduced in numbers during the fermentation. Fewer than eight viruses per 10,000 added in the first chamber were recovered in the output over a 5-day interval, 78% of these during the first 36 hours of operation. If the output was allowed to accumulate over the 5-day period, the total viable numbers in the product would be reduced another hundred-fold. With initial inocula of 10^8 to 10^{10} viruses/g total material in the first chamber, the numbers of viable viruses decreased 90% per time interval given: 18.1 hours for ZIK/1 (1-RNA, 22.5 nm smooth icosahedron), 15.6 hours for ZJ/2 (1-DNA, 5.5 X 850 nm filament), 8.3 hours for ØX174 (30 nm icosahedron with 12 large apical capsomers), 11.7 hours for PL-1 (2-DNA, 63 nm icosahedral head with 275 nm noncontractile tail), and less than 5 minutes for total kill of Ø6 (2-RNA, 60 nm icosahedron with lipid envelope). Controls (1/4 strength autoclaved FLWL) were essentially stable for the 5-day period.

Publications:

WEINER, B. A. Semicontinuous fermentation of swine waste-corn by feedback inoculum to diminish coliform numbers. In Proceedings, 4th International Symposium on Livestock Wastes. American Society of Agricultural Engineers, St. Joseph, Missouri. 1980.

Other Reports:

WEINER, B. A. Semicontinuous fermentation of swine waste-corn by feedback inoculum to diminish coliform number. Presented at 4th International Symposium on Livestock Wastes, American Society of Agricultural Engineers, Amarillo, Texas, April 15-17, 1980.

HRUBANT, G. R. Kill of Escherichia coli, Mycobacterium paratuberculosis, and bacterial viruses in the continuus fermentation of corn with feedlot waste liquid (Preliminary report). Presented at S-139 Technical Committee meeting, Gainesville, Florida, February 10-11, 1980.

HRUBANT, G. R. Kill of Mycobacterium paratuberculosis and bacterial viruses in the continuos fermentation of corn with feedlot waste liquid. Presented at NC-93 Technical Committee meeting, Lincoln and Clay Center, Nebraska, November 8-10, 1980.

E. TECHNOLOGIES FOR INDUSTRIAL USES FOR
PLANT AND ANIMAL PRODUCTS

1. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. W. Dstroy)
See Northern Agricultural Energy Center, A.2.
2. Innovative Fermentation Technology for Alcohol Production (R. W. Dstroy)
See Northern Agricultural Energy Center, A.3.

F. TECHNOLOGIES AND PRODUCTS TO INCREASE
EXPORTS OF AGRICULTURAL PRODUCTS

1. Soybean Foods of the Traditional Oriental Type for the Export Market (H. L. Wang)
 - a. Specific Objective: Evaluate methods to inactivate heat-resistant spores in soybeans.

Progress: It was previously reported that geographical location influenced internal bacterial contamination of soybeans. Moisture content also affects the amount of internal bacterial contamination. Soybeans with a moisture conent of 7.5 to 8.3% showed low contamination (5-7%), while those with increased moisture levels of 10.7 to 15.6% showed internal bacterial contamination of 16 to 26%. In investigating presoaking as a way to reduce heat-resistant spores in making soybean foods, a spore preparation of B. coagulans was added to the soaking water of soybeans. Direct microscopic observation showed a reduction of the total number of spores (viable and nonviable) after 12 hours of soaking. Soaking provides

conditions that enhance germination of the spores to vegetative forms which are easily eliminated by heating. Spores of B. coagulans were found to require a 20-minute boiling period to inactivate 96% of the viable spores. However, when soybean milk was made from soybeans soaked overnight in water with B. coagulans spores added, 96.6% of the spores were inactivated after only a 10-minute boiling period.

- b. Specific Objective: Investigate the factors affecting the quality and quantity of tofu production.

Progress: Calcium and magnesium salts are commonly used in coagulating soybean proteins to make tofu. Ionic concentrations of these salts affected the curd volume, weight, and solids recovery. Among the three salts tested (calcium chloride, magnesium sulfate and magnesium chloride), magnesium sulfate resulted in the curd with the greatest volume and weight. When the final concentrations of these salts in the soybean milk, which was made from a water:bean ratio of 10:1, were 0.006-0.008 M or 0.08-0.10 M, good curd formation, high yield, volume, and solids recovery were noted. Lowest curd weight, volume, and solids recovery were found at salt concentrations of 0.02 to 0.05 M. The percentage of solids recovery was from 82 to 102% depending on the salt concentration, and was also affected by the solid concentration of the soybean milk. Optimal boiling time for soybean milk was 15 minutes and optimal temperature for coagulation was 60°-70°C.

- c. Specific Objective: Investigate the factors affecting the production of aflatoxin in soybean substrate by Aspergillus parasiticus.

Progress: It was previously reported by other investigators that soybeans are a poor substrate for aflatoxin production by Aspergillus parasiticus, and low Zn availability was attributed as the cause. Our study showed that the addition of whole soybean meal or water extract of soybean meal to a synthetic medium stimulated growth as well as aflatoxin production by A. parasiticus. In solid substrate fermentation, the addition of 35% water instead of the 25% water added by other investigators greatly increases the aflatoxin production comparable to that of rice substrate. Furthermore, A. parasiticus grown in soybeans produced 4-5 times more G₁ than that grown in rice. Studies with synthetic media also indicated that magnesium had a significant effect on growth as well as on aflatoxin production by A. parasiticus. Both Zn and Fe showed slight increase in growth and aflatoxin production.

- d. Specific Objective: Study on a pink pigment from soybeans and soybean tempeh.

Progress: The pink pigment occasionally observed in tempeh, tofu, and bean sprouts was found to result from contamination by

Rhodotorula sp. of yeast. The source of contamination was most likely from air.

Publications:

HESSELTINE, C. W. AND H. L. WANG. The Importance of Traditional Fermented Foods. Bioscience 30 (1980):402-404.

HESSELTINE, C. W. AND H. L. WANG. Fermented Foods. Food Trade Rev. 50 (1980):473-479. Ibid 543-545.

WANG, H. L., E. W. SWAIN, AND C. W. HESSELTINE. Phytase of Molds Used in Oriental Food Fermentation. J. Food Sci. 45 (1980):1262-1266.

Other Reports:

WANG, H. L. Use of Microbial Cultures to Increase the Safety, Shelf-life, and Nutritive Value of Food Products: Legume and Cereal Products. Presented at Institute of Food Technologists meeting, New Orleans, Louisiana, June 8-11, 1980.

HESSELTINE, C. W. Future of Fermented Foods. Presented at International Fermentation Symposium, London, Ontario, Canada, July 19-26, 1980.

WANG, H. L. Nutritive Value of Fermented Foods. Presented at International Fermentation Symposium, London, Ontario, Canada, July 19-26, 1980.

HESSELTINE, C. W. A Microbe's View of Fermentation. Presented at Society for Industrial Microbiology, Flagstaff, Arizona, August 11, 1980. (Thom award address.)

HESSELTINE, C. W. The Northern Regional Research Center's Culture Collection. Presented at Soycrafters meeting, Urbana, Illinois, July 12-13, 1980.

HESSELTINE, C. W. Fermented Soymilk Products. Presented at Soycrafters meeting, Urbana, Illinois, July 9-13, 1980.

G. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Germ Plasm Bank of Microorganisms for Research on Microbial Toxins
(T. G. Pridham)
 - a. Specific Objective: Continue operation of the Agricultural Research Culture Collection (NRRL) including original and supportive research.

Progress: Mycology staff members of the Agricultural Research Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1981, the Collection maintained 72,483 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1980, the Collection distributed 3,348 strains, of which 2,271 were provided to investigators in the United States and 1,077 were sent abroad. Of 192 strains deposited in the patent collection, 130 were from foreign sources; 225 patent strains were distributed to United States researchers and 193 to foreign. Three-hundred-ninety-seven strains of molds and yeasts were identified to species for interested parties.

- b. Specific Objective: Continue study of the occurrence of bacteria, molds, and actinomycetes on and in seeds of various crop and related plants.

Progress: Data was accumulated with respect to the external and internal flora of seeds and plant parts primarily to develop methodology for searching for Actinomycetales in nonlegume nitrogen-fixing plants. The presence of molds and common bacteria continue to represent an obstacle to successful isolation work.

- c. Specific Objective: Develop media and evaluate methodology to stimulate increased sporulation by strains of molds in order to preserve abundant germ plasm by lyophilization.

Progress: Ten media were newly devised and evaluated for stimulating sporulation of 51 nonsporulating or poorly sporulating strains of molds in the NRRL collection. These strains represented species in 32 different genera. Sporulation was stimulated for eight strains representing different genera. A strain of Myxotrichum that had been in the collection for 10 years was successfully lyophilized for the first time although repeated attempts to produce spores have been made. Stock of lyophil ampules of the other seven strains were successfully replenished. One strain of Emericellopsis had not been successfully lyophilized since 1959, a strain of Spegazzinia since 1960, and a strain of Alternaria since 1961.

Publications:

BURMEISTER, H. R., J. J. ELLIS, and R. F. VESONDER. Survey for Fusaria That Produce An Antibiotic That Causes Conidia of Penicillium digitatum to Swell. Mycopathologia. Accepted.

ROGOSA, M., R. H. LAWSON, and M. I. KRICHESKY (eds.). National Work Conference on Microbial Collections of Major Importance to Agriculture. Phytopathology. In press.

WALLEN, L. L., A. J. LYONS, and T. G. PRIDHAM. Antimicrobial Activity of Patulin Derivatives: A Preliminary Report. *J. Antibiot.* 33:767-769. 1980.

WICKLOW, D. T. Biogeography and Conidial Fungi. In *The Biology of Conidial Fungi*, Vol. I, G. Cole and B. Kendrick, eds. Academic Press, New York and London. 1981. In press.

WICKLOW, D. T. Microbial Ecology. A Conceptual Approach, J. M. Lynch and N. J. Poole, eds., Blackwell Scientific Pub., Oxford, 266 pp. 1979. *Amer. Scientist* 68:570-571. 1980. (Book Review)

WICKLOW, D. T., and G. C. CARROLL, eds. *The Fungal Community: Its Organization and Role in the Ecosystem*. Marcel Dekker, New York and Basel. 1981. In press.

WICKLOW, D. T., and D. H. YOCUM. Fungal Species Number and the Decomposition of Rabbit Feces. *Trans. Brit. Mycol. Soc.* 1981. In press.

WICKLOW, D. T., and D. H. YOCUM. Effect on Larval Grazing by Lycoriella mali (Diptera:Sciaridae) on the Species Abundance of Coprophilous Fungi. *Trans. Brit. Mycol. Soc.* 1981. In press.

Other Reports:

PRIDHAM, T. G. Let's talk It Out. Radio Talk Show on Recombinant DNA Strains of Microorganisms, Radio Station WPEO, Peoria, Illinois, January 4, 1980.

PRIDHAM, T. G. Radio Interview on Culture Collection and Recombinant DNA Strains, Radio Station KMOX, St. Louis, Missouri, April 8, 1980.

WICKLOW, D. T. Fungal Communities and Ecological Ideas. Presented at University of Michigan, Ann Arbor, Michigan, January 30, 1980.

WICKLOW, D. T. Fungal Communities and Ecological Ideas. Presented at Eureka College, Eureka, Illinois, April 22, 1980.

WICKLOW, D. T. Fungal Communities and Ecological Ideas. Presented at Western Illinois University, Macomb, Illinois, April 23, 1980.

2. Aflatoxin and Other Mycotoxins in Corn and Other Cereal Grains (O. L. Shotwell)
 - a. Specific Objective: Study regions where there are mycotoxin problems in grains and investigate methods of sample handling after collection.

Progress: Zearalenone, aflatoxin, or ochratoxin were not detected in 99 Virginia wheat samples from the 1980 crop collected by the Federal Grain Inspection Service (FGIS) in the continuing survey to determine yearly differences in mycotoxin problems. In the same study, corn samples were also collected by FGIS in 1980 from Virginia and are being analyzed. Two equivalent sets of ear corn samples were collected from 57 Georgia fields in 1979. The average aflatoxin level in the set of samples dried in Georgia immediately after collection was 36 p.p.b. The average level in the set sent to NRRC before drying was 78 p.p.b. Aflatoxin levels increased during shipment. Six equivalent sets of Georgia ear corn samples were collected in 1980 to (1) study aflatoxin formation in samples after collection, (2) determine effectiveness of drying in prevention of toxin formation, and (3) investigate use of propionic acid in inhibiting aflatoxin formation after sample collection.

- b. Specific Objective: Continue study on inhalation exposure of agricultural workers to contaminated dust from aflatoxin-containing corn with Dr. William Burg, Department of Environmental Safety, University of Cincinnati.

Progress: Dust samples collected as aflatoxin-contaminated corn (240 p.p.b.) was being moved between Butler bins at the NRRC facility at Trivoli, Illinois, had a range of total aflatoxin between 13.6 and 245.9 p.p.b. with an average level of 162.6 p.p.b. Only 17% of the dust particles were less than 7 microns and would be absorbed in the lungs. Respiratory protection against the larger particles would be relatively easy. Aflatoxin analyses have been completed on dust and corn samples collected in 1979 at an elevator and three farms in the Southeast. The average aflatoxin level in settled dust samples collected at seven locations in the elevator was 222 p.p.b. The average aflatoxin level in airborne dust samples collected as trucks were delivering corn to and unloading corn from the elevator was 173 p.p.b. The average level in corn being delivered and unloaded was 78 p.p.b. The aflatoxin levels in bulk corn samples from three farms were 18, 37, and 406 p.p.b. Aflatoxin levels in airborne dust samples collected during harvest at the same three farms were 16, 21, and 114 p.p.b.

- c. Specific Objective: Develop rapid and quantitative methods of assaying corn, wheat, and grains.

Progress: The method developed for analyzing aflatoxins in animal tissues was modified for application to mixed feeds. At least five different feeds are being tested for recoveries, and naturally contaminated feeds containing corn are being analyzed by the modified animal tissue method.

- d. Specific Objective: Develop methods for determining aflatoxins and ochratoxins in animal tissues.

Progress: The method for determining aflatoxins in animal tissues was used to investigate tissues from a steer fed feed dosed with subacute toxic levels of aflatoxin (52.5 mg B₁ equivalents total, 0.3 mg/kg bodyweight) (in cooperation with A. C. Pier and J. L. Richard, National Animal Disease Center, SEA-AR, Ames, Iowa). A total of 48 ng/g of aflatoxins B₁, B₂, G₁, G₂, and M₁ were found in the liver. Aflatoxins B₁ and M₁ were the predominant toxins present at levels of 25.5 ng/g and 14.3 ng/g, respectively. Four subsections (<100 g) of the liver taken from the outer edges had less toxin content; however, the remainder of the liver showed a uniform distribution of aflatoxins. Aflatoxins were found also in the heart (16 ng/g), spleen (18.5 ng/g), skeletal muscle (flank) (12.9 ng/g), and kidney (146 ng/g). The higher toxin content of kidney tissue was unusual because aflatoxin M₁ contributed 105.5 ng/g to the total. This result suggests the possibility that B₁ is converted to M₁ in the kidneys of bovine. A total of 1.5-2% of the aflatoxin fed to the steer could be accounted for in the tissues assayed; however, several organs, blood, and waste material were not included in this study.

- e. Specific Objective: Deoxynivalenol will be isolated from field-inoculated corn and reference standards will be prepared.

Progress: Deoxynivalenol was isolated for use as a reference standard from corn that had been field-inoculated with Fusarium isolates from ear and stalk rot at the University of Illinois. Deoxynivalenol was purified by preparative high-pressure liquid chromatography (HPLC). A compound structurally related to deoxynivalenol was isolated from the inoculated corn. The compound has been partially characterized by gas chromatography (GC), mass spectrometry (MS), and gas chromatography-mass spectrometry (GC-MS) of the compound and its trimethylsilyl ether derivative and is dihydroxy-12,13-epoxytrichothec-9-ene-one, a previously unreported trichothecene. Separation parameters for deoxynivalenol and the new trichothecene were determined on RP C-8 analytical columns using ultraviolet detection at 224 nm.

Publications:

BENNETT, G. A., O. L. SHOTWELL, and C. W. HESSELTINE. Destruction of Zearalenone in Contaminated Corn. *J. Am. Oil Chem. Soc.* 57:245-247. 1980.

BURG, W. R., O. L. SHOTWELL, and B. SALTZMAN. Measurements of Airborne Aflatoxins During Handling of Contaminated Corn. *Am. Ind. Hyg. Assoc. J.* 42 (1981):1-11.

DAVIS, N. D., J. W. DICKENS, R. L. FREIE, P. B. HAMILTON, O. L. SHOTWELL, and T. D. WYLLIE. Protocols for Surveys, Sampling, Post-Collection Handling, and Analysis of Grain Samples Involved in Mycotoxin Problems. *J. Assoc. Off. Anal. Chem.* 63:95-102. 1980.

HESSELTINE, C. W., R. T. ROGERS, and O. L. SHOTWELL. Aflatoxin and Mold Flora in North Carolina in 1977 Corn Crop. *Mycologia*. In press.

SHANNON, G. M., O. L. SHOTWELL, A. J. LYONS, D. G. WHITE, and G. GARCIA-AGUIRRE. Laboratory Screening for Zearalenone Formation in Corn Hybrids and Inbreds. *J. Assoc. Off. Anal. Chem.* 63:1275-1277. 1980.

SHOTWELL, P. L. Aflatoxin in Corn--Rapid Screening Method. In 1980 Revisions to Approved Methods. *Methods of the American Association of Cereal Chemists*, 45-35:1-4. 1980.

SHOTWELL, O. L., G. A. BENNETT, M. L. GOULDEN, R. D. PLATTNER, and C. W. HESSELTINE. Survey for Zearalenone, Aflatoxin, and Ochratoxin in U.S. Grain Sorghum from 1975 and 1976 Crops. *J. Assoc. Off. Anal. Chem.* 63:922-926. 1980.

SHOTWELL, O. L., G. A. BENNETT, M. L. GOULDEN, G. M. SHANNON, R. D. STUBBLEFIELD, and C. W. HESSELTINE. Survey of 1977 Midwest Corn at Harvest for Aflatoxin. *Cereal Foods World* 25:12, 14. 1980.

SHOTWELL, O. L., M. L. GOULDEN, J. W. DICKENS, and C. W. HESSELTINE. Aflatoxin: Distribution in Contaminated Corn Plants. *Cereal Chem.* 57:206-208. 1980.

SHOTWELL, O. L., and C. W. HESSELTINE. Use of Bright Greenish-Yellow Presumptive Test for Aflatoxin in 1978 Corn. *Cereal Chem.* In press.

SHOTWELL, O. L., and C. E. HOLADAY. Minicolumn Detection Methods for Aflatoxin in Raw Peanuts: Collaborative Study. *J. Assoc. Off. Anal. Chem.* In press.

STUBBLEFIELD, R. D. Stability and Molar Absorptivity of Aflatoxin M_1 in Acetonitrile-Benzene (1+9). *J. Assoc. Off. Anal. Chem.* 63:634-636. 1980.

STUBBLEFIELD, R. D., H. P. VAN EGMOND, W. E. PAULSCH, and P. L. SCHULLER. Determination and Confirmation of Identity of Aflatoxin M_1 in Dairy Products: Collaborative Study. *J. Assoc. Off. Anal. Chem.* 63:907-921. 1980.

VAN EGMOND, H. P., and R. D. STUBBLEFIELD. Improved Method for the Confirmation of Identity of Aflatoxins B_1 and M_1 in Dairy Products and Animal Tissues. *J. Assoc. Off. Anal. Chem.* In press.

Other Reports:

BENNETT, G. A. Methods of Zearalenone Analysis. Presented at the Association of Official Analytical Chemists' Spring Workshop, St. Louis, Missouri, April 7-10, 1980.

BENNETT, G. A. Research on Fusarium Mycotoxins: Zearalenone and Deoxynivalenol. Presented at NC-129 Mycotoxin Committee meeting, Champaign, Illinois, April 21-22, 1980.

BENNETT, G. A. Ethanol Production from Zearalenone-Contaminated Corn. Presented at the International Society for Fat Research/American Oil Chemists' Society World Congress, New York, New York, April 27-May 1, 1980.

BENNETT, G. A. Associate Referee Report on Zearalenone. Presented at Association of Official Analytical Chemists' meeting, Washington, D.C., October 19-23, 1980.

SHOTWELL, O. L. BGY Presumptive Test. Presented at Association of Official Analytical Chemists' Spring Workshop, St. Louis, Missouri, April 7-10, 1980.

SHOTWELL, O. L. Aflatoxin: Analysis of Contaminated Corn Dust. Presented at American Industrial Hygiene Conference, Houston, Texas, May 20-23, 1980.

SHOTWELL, O. L. Mycotoxin Update. Presented at American Corn Dry Milling Conference, Northern Regional Research Center, Peoria, Illinois, June 3-4, 1980.

SHOTWELL, O. L. Minicolumn Detection Method for Aflatoxin in Raw Peanuts: Collaborative Study. Presented at Association of Official Analytical Chemists' meeting, Washington, D.C., October 19-23, 1980.

SHOTWELL, O. L. Aflatoxin in Corn: Occurrence, Formation, and Analysis. Presented at Coloquio Internacional sobre Conservacion de Semillas y Granos, Oaxtepec, Morelos, Mexico, October 20-26, 1980.

STUBBLEFIELD, R. D. Led Panel Discussion on High Pressure Liquid Chromatography of Mycotoxins at the Association of Official Analytical Chemists' Spring Workshop, St. Louis, Missouri, April 7-10, 1980.

STUBBLEFIELD, R. D. Aflatoxin M₁ Research at the Northern Regional Research Center. Presented at the NC-129 Mycotoxin Committee meeting, Champaign, Illinois, April 21-22, 1980.

STUBBLEFIELD, R. D. The Determination of Aflatoxin in Animal Tissue. Presented at Association of Official Analytical Chemists' meeting, Washington, D.C., October 19-23, 1980.

3. Metabolites of Toxin-Producing Fungi Found in Corn and Other Cereal Grains (M. D. Grove)

- a. Specific Objective: Elucidate reaction pathways of the coumarin ring system of aflatoxin upon inactivation by ammoniation.

Progress: Ammonia-induced decomposition of an aflatoxin-like model ketocoumarin proceeds via two temperature-dependent pathways. At 50°C the major product is a ketophenol formed by lactone ring opening followed by decarboxylation. At 37°C, in addition to the ketophenol, an approximately equal amount of an enolized alphadiketone is formed presumably by oxidation and decarboxylation. The diketone, which is not formed from the ketophenol, is further degraded through loss of the cyclopentandione ring to 3,5-dimethoxyphenol. While this phenol is stable to ammonia, in an ammoniated corn matrix it undergoes further reaction.

- b. Specific Objective: Determine the reactivity of the dihydrofuran portion of aflatoxin under conditions used for ammoniation of corn.

Progress: Synthesis of the prerequisite 6-methoxy-3a,8a-dihydrofuro-[2,3-b]-benzofuran was initiated. Four steps of the six-step synthetic sequence have been carried out to give 2-hydroxy-6-ethoxy-2,3,3a,8a-tetrahydrofuro-[2,3-b]-benzofuran, and procedures were developed for purification of intermediates.

- c. Specific Objective: Expand studies to develop methods for production of toxic metabolites of Penicillium viridicatum for animal toxicity studies.

Progress: Fermentation studies on the production of viridicatumtoxin, a yellow polycyclic metabolite of a South African strain of P. viridicatum, were initiated. Column and HPLC separation techniques were developed which enabled isolation of the crystalline toxin. Identity of the compound was confirmed by spectroscopic means. A sample was furnished by Dr. W. Carlton, SAES, Purdue University, for preliminary toxicological evaluation.

- d. Specific Objective: Correlate xanthomegnin production with elaboration of other mycotoxins by various strains of P. viridicatum.

Progress: Thirty-six strains of P. viridicatum were grown on rice, and extracts appropriate for analysis were prepared from the cultures. An HPLC method was developed for the quantitative determination of xanthomegnin and viomellein in these extracts.

- e. Specific Objective: Study Fusarium strains in the Agricultural Research Culture Collection (NRRL) for their potential to produce metabolites causing animal feeds to be unpalatable.

Progress: Nineteen of 40 culture extracts of Fusarium strains rejected by mice contained T-2 toxin and 3 contained vomitoxin, both known refusal factors. However, several strains of species not known to produce trichothecenes were found that cause the refusal response in mice. Mice readily drank aqueous solutions of moniliformin or butenolide at a concentration of 0.5 mg/ml. After 21 days, the mice were visibly unaffected, except for a slightly reduced weight gain. An average daily amount of moniliformin equivalent to nearly three times a single oral LD₅₀ for each of 21 days was consumed. Butenolide was consumed at nearly one-half the single oral LD₅₀ each day without obvious effect. These results indicate the two mycotoxins are not nearly as lethal when consumed gradually as compared to a single large dose.

- f. Specific Objective: Study production and identify antibiotics produced by toxic Fusarium strains and determine their effect on overall toxicity or feed refusal.

Progress: An antifungal antibiotic produced by 23 of 132 Fusarium strains was identified as a hexapeptide with an alkyl chain. The antibiotic was not refused by mice when added to their drinking water and was not toxic to mice by intraperitoneal injection. Equisetin, an antibiotic produced by F. equiseti, was found by the National Cancer Institute (NCI) to be active against the in vivo system 3PS 31. In response to a request by NCI, 8.7 g of equisetin was isolated and furnished for additional testing. Because HT-2 toxin has shown a good spectrum of antitumor activity and additional compound was required by NCI for xenografts (human tumor lines in athymic mice), a sample of HT-2 toxin was supplied.

- g. Specific Objective: Assess mycotoxicoses of farm animals thought to be caused by consumption of moldy grains or feeds made from infected grains.

Progress: Vomitoxin was found in feed that was presumed to cause tissue to appear in the stool of chickens. The possible role of vomitoxin in this syndrome is being studied. T-2 toxin was provided Dr. P. Hamilton, SAES, North Carolina State University, to study its effect on specific sites of the blood coagulation mechanism. T-2 toxin produces a distinct coagulopathy in chickens characterized by a primary effect on coagulation Factor VII activity and secondary effects on prothrombin and fibrinogen. These effects provide further evidence for a possible role of T-2 toxin in alimentary toxic aleukia. Moniliformin and an assayed moniliformin-containing culture were provided Dr. N. Allen, SAES, University of Minnesota, for chicken feeding studies. Chickens, like mice, were found to

tolerate relatively large amounts of the toxin when it was added to their feed.

- h. Specific Objective: Study detoxification of cereals containing the refusal factor, vomitoxin.

Progress: Sulfur dioxide treatment of corn containing vomitoxin for different lengths of time and concentration did not significantly decrease the refusal response in a swine bioassay test.

- i. Specific Objective: Continue study with the University of Illinois, College of Veterinary Medicine, of mycotoxicoses in equine.

Progress: Intravenous administration of moniliformin to a healthy female donkey at 1 mg/kg bodyweight for 26 days resulted in death with no premonitory signs. No gross neural lesions were noted, but microscopic lesions within the cerebrum consisted of perivascular hemorrhage, satellitosis, and neurophagia. These lesions, although relatively mild, are similar to those seen in both naturally occurring and experimentally produced cases of equine leucoencephalomalacia (ELEM). A possible role of secalonic acid in ELEM was investigated in small animals. No neural lesions were observed in rabbits fed with secalonic acid. Its poor solubility properties precluded intravenous administration to donkeys. The intraperitoneal LD₅₀ of secalonic acid in mice ranged from 26.5 to 51.7 mg/kg dependent on strain and sex. Secalonic acid was nontoxic and nonteratogenic to chick embryos and exhibited weak antibiotic properties.

- j. Specific Objective: Determine the fate of two trichothecenes in swine.

Progress: Several hundred milligrams each of vomitoxin and T-2 toxin were provided the University of Illinois, College of Veterinary Medicine, for development of assay procedures to study toxin distribution in body tissues and excretion patterns in swine.

Publications:

BUCK, W. B., J. C. HALIBURTON, J. P. THILSTED, T. F. LOCK, and R. F. VESONDER. Equine Leucoencephalomalacia: Comparative Pathology of Naturally Occurring and Experimental Cases. In Amer. Assoc. Vet. Diagnosticians Proc. 239-258. 1979.

BURMEISTER, H. R., M. D. GROVE, and W. F. KWOLEK. Moniliformin and Butenolide: Effect on Mice of High-Level, Long-Term Oral Intake. Appl. Environ. Microbiol. 40:1142-1144. 1980.

BURMEISTER, H. R., R. F. VESONDER, and W. F. KWOLEK. Mouse Bioassay for Fusarium Metabolites: Rejection or Acceptance When Dissolved in Drinking Water. Appl. Environ. Microbiol. 39:957-961. 1980.

CIEGLER, A., H. R. BURMEISTER, R. F. VESONDER, and C. W. HESSELTINE. Mycotoxins: Occurrence in the Environment. In *Mycotoxins and N-Nitroso Compounds*, Chemical Rubber Company Press, Boca Raton, Florida. In press.

CIEGLER, A., A. W. HAYES, and R. F. VESONDER. Production and Biological Activity of Secalonic Acid D. *Appl. Environ. Microbiol.* 39:285-287. 1980.

HUFF, W. E., J. A. DOERR, P. B. HAMILTON, D. D. HAMANN, R. E. PETERSON, and A. CIEGLER. Evaluation of Bone Strength During Aflatoxicosis and Ochratoxicosis. *Appl. Environ. Microbiol.* 40:102-107. 1980.

VESONDER, R. F., and C. W. HESSELTINE. Vomitoxin: Natural Occurrence on Cereal Grains and Significance as a Refusal and Emetic Factor to Swine. *Process Biochem.* In press.

WALLEN, L. L. Aflatoxin. In *Yearbook of Science and Technology*. S. P. Parker, ed., McGraw-Hill Co., New York, New York, pp. 91-93. 1980.

WALLEN, L. L., A. J. LYONS, and T. G. PRIDHAM. Antimicrobiol Activity of Patulin Derivatives: A Preliminary Report. *J. Antibiot.* 33:767-769. 1980.

Other Reports:

GROVE, M. D. Production of Penicillium viridicatum Pigments. Presented at NC-129 Mycotoxin Committee meeting, Champaign, Illinois, April 21-22, 1980.

PETERSON, R. E. Prep 500 Purification of Several Mycotoxins. Presented at Waters Associates Symposium on High Performance Liquid Chromatography Analysis of Vitamins and Toxins, Oak Brook, Illinois, April 17-18, 1980.

VESONDER, R. F. Etiology of Equine Leucoencephalomalacia and Fusarium Toxins as Swine Refusal Factors. Presented at NC-129 Mycotoxin Committee meeting, Champaign, Illinois, April 21-22, 1980.

VESONDER, R. R. Swine Refusal Factors Elaborated by Fusarium Strains and Identified as Trichothecenes. Presented at American Phytopathological Society meeting, Minneapolis, Minnesota, August 24-28, 1980.

4. Origin and Ecology of Mycotoxin-Producing Fungi in Grain (D. T. Wicklow)

- a. Specific Objective: Examine stored materials from an ancient rodent cache economy for presence of mycotoxins.

Progress: Mycotoxicogenic species of Aspergillus and Penicillium were among the prevalent molds recorded from external cheek pouches and subterranean seed caches of Sonoran Desert kangaroo rats. Direct microscopic observations of seeds and floral parts retrieved from rodent burrows revealed the presence of conidial apparatus (A. ostianus, A. flavus, P. cyclopium, P. griseofulvin), evidence that burrow environments allow for the growth of these molds. All isolates of A. flavus cultured on cracked corn (8d; 28°C) produced substantial quantities of aflatoxins (>1,800 p.p.b. B₁), but no aflatoxins were detected in samples of rodent-stored seeds. No isolate of A. ostianus tested produced ochratoxins or citrinin on cracked corn. Because the rodent-cache mold community also includes species known to prevent aflatoxin synthesis by A. flavus (A. niger group) or degrade aflatoxin (Rhizopus spp.), our working hypothesis is that rodents reduce the chances of aflatoxin contamination by maintaining populations of these fungal competitors.

- b. Specific Objective: Compare the alkaloids in sclerotia of A. flavus and A. parasiticus strains to assess their significance in distinguishing isolates at species level.

Progress: Aflatoxins and cyclopiazonic acid are reported for the first time from sclerotia of A. flavus, particularly in sclerotia of isolates from warmer latitudes. The fungal sclerotium is an important survival structure in the life history of sclerotium-producing fungi, and we are interested in the possible role of sclerotial mycotoxins in chemical defense.

- c. Specific Objective: Examine the discriminating value of ultraviolet-induced fluorescence in APA test medium [Czapek's solution agar containing corn steep liquor, HgCl₂ and (NH₄)₂HPO₄ instead of NaNO₃] when distinguishing among aflatoxin-producing and nonproducing strains of A. flavus isolated from cereals.

Progress: Aflatoxin-producing ability (APA) medium was tested for its ability to distinguish aflatoxin-positive from aflatoxin-negative strains of A. flavus in naturally occurring populations of corn at harvest. All of the aflatoxin-positive strains and some of the aflatoxin-negative strains produced aflatoxins when cultured on cracked corn. While the data indicate that APA medium is not entirely reliable in distinguishing potential aflatoxin-producing strains of A. flavus from nontoxigenic strains, it is significant that the medium did not yield false positives.

Publications:

DORNER, J. W., R. J. COLE, J. P. SPRINGER, R. H. COX, H. CUTLER, and D. T. WICKLOW. Isolation and Identification of Two New Biologically Active Norditerpene Dilactones from Aspergillus wentii. Phytochemistry 19:1157-1161. 1980.

DORNER, J. W., R. J. COLE, R. HILL, D. T. WICKLOW, and R. H. COX. Penicillium rubrum and Penicillium biforme, New Sources of Rugulovasines A and B. Appl. Environ. Microbiol. 40:685-687. 1980.

WICKLOW, D. T., C. W. HESSELTINE, O. L. SHOTWELL, and G. L. ADAMS. Interference Competition and Aflatoxin Levels in Corn. Phytopathology 70:761-764. 1980.

WICKLOW, D. T., G. SHANNON, and O. L. SHOTWELL. Production of Sterigmatocystin by Aspergillus versicolor QM 432 Used in Fungus Resistance Tests for United States Military Specifications. Mycopathologia 71:37-39. 1980.

WICKLOW, D. T., O. L. SHOTWELL, and G. L. ADAMS. Further Observations on the Use of APA Medium to Distinguish Aflatoxin-Producing Strains of Aspergillus flavus. Appl. Environ. Microbiol. In press.

5. Microbial Species Interactions and Development of Aflatoxin in Preharvest Corn (Cooperative Agreement - University of Wisconsin)

- a. Specific Objective: Contrast aflatoxin accumulation in corn grown in the Biotron under a climate regimen typical of upper midwest (aflatoxin rare) vs. southeastern U.S. (aflatoxin common).

Progress: Our first objective was to grow various commercial corn hybrids to maturity (dent kernels) in a specially modified plant growth room. Mature corn ears had never been produced during a 15-year history of Biotron research programs. We were successful in obtaining 80-98% fertility (mature kernels) on ears produced by several widely distributed commercial hybrids when grown under accurately defined (Biotron) conditions. Selecting these hybrids for further study, we next examined methods of introducing A. flavus inoculum (as spray on silks; on toothpicks used to wound kernels) and the significance of drought stress in A. flavus establishment and aflatoxin contamination. Visual observation shows A. flavus sporulation on silks and wounded kernels. At this writing, the ears had not reached full dent and, therefore, were not yet available for analysis. It is critical that we first create growth room environments leading to A. flavus infection and aflatoxin development before we introduce mold competitors in the experimental design.

6. Establishment and Identification of Toxic Metabolites of Certain Molds and Aflatoxin in Livestock Fodder (P.L. 480 Grant - Institute for Animal Nutrition and Feed Technology, Zagreb University)

b. Specific Objective: Identification and measurement of mycotoxins in corn.

Progress: Much time was spent on the effect of ochratoxin in feed on the lipid and protein content of chickens. At levels of 5.0 p.p.m. ochratoxin, the total lipids and total proteins were reduced. At 0.5 p.p.m., however, the total lipids and proteins in blood was increased over controls. A second study deals with the contamination of corn with mycotoxins during the very wet year of 1978-1979. Zearalenone was found in 55% of the 116 samples, ochratoxin in 64%, skin-irritating factors in 10%, and aflatoxin in 2.6%. The chief molds present were Fusarium and Penicillium. Zearalenone was found as high as 275 mg/kg. A further study was made of the molds in corn in the area where the nephropathic disease is found in humans versus a nonnephropathic area, but no difference in the mold flora was found. However, the levels of ochratoxin and zearalenone were higher in the nephropathic area in corn than in the areas where disease in humans is not found.

Publications:

PEPELJNJAK, S., and I. BALZER. A Survey of Mycological and Mycotoxicological Researches in the Nephropathic and Anephropathic Areas of Yugoslavia (Croatia). In press.

PEPELJNJAK, S., and I. BALZER. Konzerviranje Kukuruza uz Dodatak 0,0-Dimetil,2,2,-Diklorvinilfosfata. In press.

BALZER, I., S. PEPELJNJAK, and S. CUTURIC. The Contamination of Corn with Mycotoxins in Yugoslavia in the Year 1978-1979. In press.

BALZER, I., S. CUTURIC, s. PEPELJNJAK. On the Tolerances of Mycotoxins in Food and Feed. Veterinaria 28:433-436. 1979.

RUPIC, V., B. LIKER, S. MUZIC, I. BOGDANIC, and I. BALZER. The Effect of Ochratoxin A in Feed on the Blood Content of Lipids and Proteins in Chickens. Arh. Hig. Rada 29:139-145. 1978.

BALZER, I., C. BOGDANIC, and S. PEPELJNJAK. Rapid Thin Layer Chromatographic Method for Determining Aflatoxin B₁, Ochratoxin A, and Zearalenone in Corn. J. Assoc. Off. Anal. Chem. 61:584-585. 1978.

BALZER, I., L. OZEGOVIC, J. TUITE, and P. SCOTT. Panel on Zearalenone. In Mycotoxins in Human and Animal Health. J. V. Rodericks, C. W. Hesseltine, and M. A. Mehlman, eds., pp. 415-416, Pathotox Publishers, Inc., Park Forest South, Illinois. 807 pp. 1977.

LIKER, B., V. RUPIC, C. BOGDANIC, I. BALZER, S. MUZIC, and M. HERCEG. Influence of Ochratoxin A on the Activity of Alkaline Phosphatase, Sorbitol Dehydrogenase, and Glutamate Dehydrogenase in Chick Blood Plasma. Veterinarski Arhiv 48:23-32. 1978.

BALZER, I., C. BOGDANIC, and S. MUZIC. Natural Contamination of Corn (Zea mais) with Mycotoxins in Yugoslavia. Ann. Nutr. Alim. 31:425-430. 1977.

RUPIC, V., B. LIKER, S. MUZIC, C. BOGDANIC, and I. BALZER. The Influence of Ochratoxin A in Feed on the Quantitative Blood Picture of Chicken. Veterinarski Glasnik 31:725-728. 1977.

BALZER, I., C. BOGDANIC, S. MUZIC, and S. PEPELJNJAK. Neki Vanjski Koji Utjecu na Zagadenje Kukuruza Plijesnima. Krmiva 19:101-104. 1977.

MUZIC, S., C. BOGDANIC, S. PEPELJNJAK, and I. BALZER. Zagadenje Kukuruza Mikotoksinima. Krmiva 19:25-28. 1977.

MUZIC, S., C. BOGDANIC, and I. BALZER. Establishment of Toxic Metabolites of Certain Moulds in Livestock Fodder. Works of the Agricultural Faculty, University of Sarajevo, Year XXIV, No. 27:531-538. 1976.

BOGDANIC, C., S. MUZIC, and I. BALZER. Mycotoxins in Livestock Fodder. Works of the Agricultural Faculty, University of Sarajevo, Year XXIV, No. 27:519-530. 1976.

HORTICULTURAL AND SPECIAL CROPS LABORATORY

L. H. Princen, Chief

Research Leaders: R. Kleiman, J. A. Rothfus, C. R. Smith, Jr., and
H. L. Tookey

A. BREEDING AND PRODUCTION OF FORAGE CROPS FOR HAY, PASTURES, AND OTHER USES INCLUDING TURF

1. Chemicals in Tall Fescue Affecting Livestock Health and Forage Utilization (S. G. Yates)

- a. Specific Objective: Continue separation, identification, and quantitation of constituents in toxic fractions from tall fescue.

Progress: All major constituents detectable by GC-MS in toxic fractions from tall fescue have been identified. Attention now focuses on isolation and characterization of the less-prominent components seen in GC-MS analyses. Separation techniques including GC and HPLC, are being developed to resolve this group of compounds, which appear to constitute about one-third of the material in toxic fractions and to consist of less volatile, more polar and, possibly, higher molecular weight substances. HPLC chromatography on a C-18 reversed phase column has proven effective in removing water-soluble components and may provide access to constituents not amenable to purification via GC.

In cooperative research (University of Kentucky), it was learned that N-formylloline and pyroglutamic acid did not affect summer syndrome in cattle.

2. Bioassay of Chemical Constituents of Tall Fescue Forage (Cooperative Agreement - University of Missouri)

- a. Specific Objective: Assay chemically defined preparations containing components identified in toxic anion fractions from tall fescue.

Progress: Tests were completed on all major and one of the minor components identified among toxic anions from fescue. Synthetic mixtures of succinic, glyceric, glycolic, shikimic, quinic, malic, lactic, pyroglutamic, and pipecolic acids can cause reddening at the coronary band, but they have yet to produce severe symptoms of fescue foot. Neither do such mixtures sustain depressed foot temperatures in the same manner as do unidentified fescue constituents that elute from ion-exchange resin in 0.05 N formic acid in 50% aqueous ethanol.

Intraperitoneal infusion of test substances and temperature measurement by videothermometry (reported previously) constitute substantial improvements in test protocol, but cattle tests still suffer from limitations and inconveniences common to large animal experiments. Goats pastured on fescue that is managed to promote toxicity, thus far show no marked response that would make them useful test animals under forage conditions. Infused goats, however, exhibit reduced foot temperatures when treated with fractions that reduce coronary band temperatures in cattle. This encouraging result may lead to a simpler investigation of reduced peripheral circulation, which occurs in fescue toxicosis. Rat populations show considerable individual variation in tail vein dilation response to temperature change. Selection for response similarity may provide groups for laboratory-scale tests of effects on temperature regulation.

Publications:

CORNELL, C. N., G. B. GARNER, B. W. DOYLE, S. G. YATES, AND J. A. ROTHFUS. The Bovine IP Bioassay as a Measure of Physiological Distress in Cattle Grazing Tall Fescue. *J. Anim. Sci.* 51, Supplement 1 (1980), Abstract #315.

GARNER, G. B., C. N. CORNELL, S. G. YATES, AND J. A. ROTHFUS. Fescue Foot Syndrome: Further Fractionation of Toxic Extracts and Testing of Synthetic Compounds via the Bovine IP Assay. *J. Anim. Sci.* 51, Supplement 1 (1980), Abstract #321.

Other Reports:

GARNER, G. B., C. N. CORNELL, S. BELL, AND S. G. YATES. Summary of a 3 year (1977-1980) fescue foot toxicity trial at Southwest Center. Southwest Missouri Center Field Day, University of Missouri, Columbus, MO, September 19, 1980.

GARNER, G. B., C. N. CORNELL, S. G. YATES, AND J. A. ROTHFUS. Fescue toxicosis - cooperative fescue foot toxin research. AES Collaborator's meeting, NRRC, Peoria, IL, October 20-21, 1980.

B. INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION, AND DOCUMENTATION OF PLANT GERMPLASM

1. Chemical Analysis of Uncultivated Plants (R. Kleiman)

- a. Specific Objective: Chemically screen seeds and characterize novel constituents in seed oils and other plant components.

Progress: Of the 66 new crop species screened for their chemical composition, a number have higher than 25% oil content. The highest of these was Croton capitatus, collected in Mississippi, with 62%

oil. Banksia paludosa had 79% protein (N X 6.25). Many indicated unusual composition by TLC and/or GLC. Of the 88 oils converted to fatty acid methyl esters and analyzed by GLC and TLC, several revealed unusual fatty acid composition. For example, four Asclepiadaceae contain 16:1, up to 18%, two Callitris species contain about 20% *cis*-5 C₂₀ acids. Cuphea petiolata, collected in Illinois, contained 76% 10:0. In-depth characterization of unusual seed oil components revealed about one-fourth of Cacalia atriplicifolia seed oil is composed of long chain esters of dammarenediol II. This triterpene has previously been found (unesterified) in the bark of some tropical trees. Cacalia is an herb collected in Illinois. A new neolignan, 5,8-epoxy-6,7-dimethyl 2',3',2",3" dimethylenedioxy-4',1"-dimethoxy-1,2:3,4-dibenz-1,3-cyclooctadiene, was isolated from the petroleum ether extract of Clerodendron inerme seed. Three alkaloids were identified in the petroleum ether extract of Bocconia arborea seed. They are dihydrosanguarine, dihydروبocconine, and dihydrochelythrine.

- b. Specific Objective: Analysis of potentially new crops in cooperation with plant breeders.

Progress: Forty-two rapeseed lines were analyzed for oil and erucic acid content. These lines were from Washington State University which is attempting the development of double zero rape for Northwest United States. Analysis of Vernonia galamensis seed grown in Puerto Rico revealed 72% and 78% vernolic acid.

- c. Specific Objective: Develop methods for analysis and characterization of plant materials.

Progress: An HPLC method was developed to separate and quantitate free acids, triglycerides, diglycerides, and monoglycerides. The method utilizes a cyano-bonded Partisil column under gradient elution conditions and an infrared detector. Mass spectrometric methods were developed for the analysis of the natural toxicant falcarinol and for mycotoxins, such as zearalenone, vomatoxin, and ochratoxin. Chemical ionization-mass spectrometry was applied to fatty acid methyl esters and wax esters.

- d. Specific Objective: Develop methods for processing Vernonia galamensis seed to produce a high-epoxy, low, free acid oil.

Progress: Laboratory and pilot plant experiments show that a relatively conventional oil extraction scheme can be used to remove oil from heat and moisture-tempered V. galamensis seed flakes. FFA content of crude oils ranged from 0.4 to 1.35%. Vernolic acid ranged from 72 to 78% in the oils (3.6 to 3.9% oxirane oxygen). The oil may be conventionally refined with minimal alkali and neutral bleaching earth.

e. Specific Objective: Evaluate agronomics of Vernonia galamensis in the U.S.

Progress: In the laboratory, V. galamensis plants show a tendency to produce flowers with decreasing day length with some viable seed produced from the flowers (self-fertile).

f. Specific Objective: Evaluate means of forming superior coatings from epoxy oils.

Progress: Vernonia oils without natural promoters (polar materials present in crude oils) polymerize slowly, not at all, or only at high temperatures. Metal octoates generally promote film formation in such oils as also do organic acids at levels of 1.5% or greater. A hydroxy acid additive gave a particularly glossy film. Experiments using traditional polyfunctional catalysts often used with commercial epoxy resins, indicated that internal epoxy groups as found in Vernonia oil are much less reactive than commercial epoxy resins.

Publications:

CARLSON, K. D., W. J. SCHNEIDER, L. H. PRINCEN, AND S. P. CHANG. Vernonia galamensis Seed Oil: A New Source for Epoxy Coatings. In Sources of Fats and Oils, Edited by E. H. Pryde and L. H. Princen, American Oil Chemists' Society, Champaign, IL (in press).

CHANG, S. P. Allyl Esters and Allyl Epoxy Esters from Crambe Oil. J. Am. Oil Chem. Soc. 56 (1979):855-856.

CHERNIAK, A. R., E. REISS, M. E. SLODKI, R. D. PLATTNER, AND S. O. BLUMER. Structure and Antigenic Activity of the Capsular Polysaccharide of Cryptococcus neoformans Serotype A. Mol. Immunol. 17 (1980):1025-1032.

CRAWFORD, C. G., R. D. PLATTNER, D. J. SESSA, AND J. J. RACKIS. Separation of Oxidized and Unoxidized Molecular Species of Phosphatidylcholine by High Pressure Liquid Chromatography. Lipids 15 (1980):91-94.

DAXENBICHLER, M. E., G. F. SPENCER, AND W. P. SCHROEDER. 3-Hydroxypropyl-glucosinolate, A New Glucosinolate in Seeds of Erysimum hieracifolium and Malcolmia maritima. Phytochemistry 19 (1980):813-815.

JACKSON, L. K., M. E. SLODKI, M. C. CADMUS, K. A. BURTON, AND R. D. PLATTNER. 3-O-Methyl-L-rhamnose from a Rhizobium Capsular Polysaccharide. Carbohydr. Res. 82 (1980):154-157.

PAYNE-WAHL, K. L. AND R. KLEIMAN. trans-2-Hexadecenoic Acid in Aster scaber Seed Oil. J. Nat. Prod. (in press).

PAYNE-WAHL, K. L., R. D. PLATTNER, G. F. SPENCER, AND R. KLEIMAN. Separation of Tetra-, Penta-, and Hexaacyl Triglycerides by High Performance Liquid Chromatography. *Lipids* 14 (1979):601-605.

PAYNE-WAHL, K. L., G. F. SPENCER, R. D. PLATTNER, AND R. O. BUTTERFIELD. An HPLC Method for Quantitation of Free Acids, Mono-, Di-, and Triglycerides Using An Infrared Detector. *J. Chromatogr.*, (in press).

PLATTNER, R. D. High Performance Liquid Chromatography of Triglycerides. In *Methods in Enzymology*, Edited by J. M. Lowenstein, Vol. 72, Academic Press (in press).

PLATTNER, R. D. High-performance Liquid Chromatography of Triglycerides: Controlling Selectivity with Reverse Phase Columns. *J. Am. Oil Chem. Soc.* (in press).

PRINCEN, L. H. AND M. A. TAYLOR. Fatty Acids in Solution. In *Fatty Acids*, Edited by E. H. Pryde, American Oil Chemists' Society, Champaign, IL, 1979, Chapter 10, pp. 195-217.

SHOTWELL, O. L., G. A. BENNETT, M. L. GOULDEN, R. D. PLATTNER, AND C. W. HESSELTINE. Survey for Zearalenone, Aflatoxin, and Ochratoxin in U.S. Grain Sorghum from 1975 and 1976 Crops. *J. Assoc. Off. Anal. Chem.* 63 (1980):922-926.

SPENCER, G. F. Dammarenediol II Esters from Cacalia atriplicifolia L. Seed Oil. *J. Nat. Prod.* (in press).

SPENCER, G. F. AND M. E. DAXENBICHLER. Gas Chromatography-Mass Spectrometry of Nitriles, Isothiocyanates, and Oxazolidinethiones Derived from Cruciferous Glucosinolates. *J. Sci. Food Agric.* 31 (1980):359-367.

SPENCER, G. F., L. W. TJARKS, AND R. KLEIMAN. Alkyl and Phenylalkyl Anacardic Acids from Knema elegans Seed Oil. *J. Nat. Prod.* 43 (1980):724-730.

VICK, B. A., D. C. ZIMMERMAN, AND D. WEISLEDER. Thermal Alteration of a Cyclic Fatty Acid Produced by a Flaxseed Extract. *Lipids* 14 (1979):734-740.

Other Reports:

CULL, I. M. Midwestern Plants for Future Crops. Illinois State Academy of Science Meeting, Lisle, IL, April 18-19, 1980.

KLEIMAN, R. AND L. H. PRINCEN. Seed Oils as a Renewable Resource for Industrial Use. FAO/IAEA Meeting, Vienna, Austria, November 17-21, 1980.

PRINCEN, L. H. Alternate Industrial Feedstocks from Agriculture.
American Institute of Chemical Engineers Meeting, Philadelphia, PA,
June 8-12, 1980.

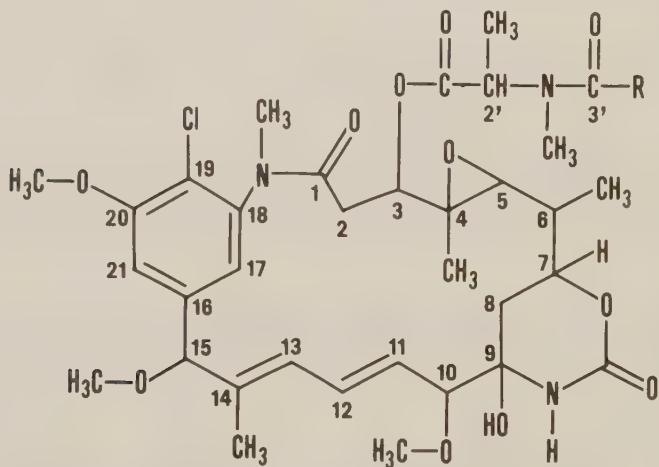
PRINCEN, L. H. Research Opportunities on New Crops in Illinois.
Illinois State Academy of Science Meeting, Lisle, IL, April 18-19,
1980.

SPENCER, G. F. NMR and Mass Spectrometry of Anacardic Acid Derivatives. ISF-AOCS Congress, New York, NY, April 27-May 1, 1980.

2. Biologically Active Plant Constituents for Pest Control and Medicine (C. R. Smith)

- a. Specific Objective: Detect, isolate, and characterize constituents of seeds and other plant parts that have potential value as pest control agents, as antitumor agents, or that have other biological activities beneficial to agriculture.

Progress: Five new antitumor maytansinoid compounds isolated from *Trewia nudiflora* seed extract have been characterized by NMR and MS; in the case of three of these compounds, structural elucidation is considered conclusive. Trewiasine, the most abundant of these maytansinoids, has the following structure [R = -CH(CH₃)₂]:



In order to characterize the remaining two maytansinoids more fully, 260 pounds of seed have been extracted; fractionation is at an advanced stage. Fractionation of the extract of Taxus wallichiana (formerly Cephalotaxus mannii) has provided two additional antileukemic taxane derivatives, for a total of five. The two newest taxanes have an N- α -methylbutyryl-3-amino-2-hydroxy-3-phenylethyl ester side chain. Extensive fractionation has been carried out on extracts of diseased peach wood that are highly attractive to the female peach borer moth. Some highly attractive fractions have been obtained, and some of their components have

been identified by GC-MS. Extensive fractionation also has been carried out on oat extracts that are attractive to the saw-toothed grain beetle (Oryzaephilus surinamensis L.). The ethanol extract of Diplocisia glaucescens seed (family Menispermaceae), found to be lethal to European corn borer larvae, has been fractionated extensively; at least a portion of this activity has been found to reside in an alkaloid which has been partially characterized. According to preliminary results from Dr. T. L. Ladd (SEA, Wooster, Ohio), neriifolin (from Thevetia thevetioides) is a good antifeedant for the Japanese beetle, a soybean pest which is moving westward. In addition, Dr. David Reed (SEA, Vincennes, Indiana) reports that neriifolin has toxic and growth-regulating properties for the codling moth.

Publications:

MC LAUGHLIN, J. L., B. FREEDMAN, R. G. POWELL, AND C. R. SMITH, JR. Neriifolin and 2'-Acetylneriifolin: Insecticidal and Cytotoxic Agents of Thevetia thevetioides Seeds. *J. Econ. Entomol.* 73 (1980):398-402.

MC LAUGHLIN, J. L., R. W. MILLER, R. G. POWELL, AND C. R. SMITH, JR. 19-Hydroxybaccatin III, 10-Deacetylcephalomannine, and 10-Deacetyltaxol: New Antitumor Taxanes from Taxus wallichiana. *J. Nat. Prod.* (in press).

MIKOLAJCZAK, K. L. AND C. R. SMITH, JR. Synthetic Cephalotaxine Esters having Antileukemic P388 Activity. U.S. Patent 4,203,966. May 20, 1980.

MILLER, R. W. A Brief Survey of Taxus Alkaloids and Other Taxane Derivatives. *J. Nat. Prod.* 43 (1980):425-437.

MILLER, R. W., R. G. POWELL, C. R. SMITH, JR., E. ARNOLD, AND J. CLARDY. Antileukemic Alkaloids from Taxus wallichiana Zucc. *J. Org. Chem.* (in press).

POWELL, R. G., R. W. MILLER, AND C. R. SMITH, JR. Cephalomannine, A New Antitumor Alkaloid from Cephalotaxus mannii. *J. C. S. Chem. Commun.* (3) (1979):102-104.

POWELL, R. G. AND C. R. SMITH, JR. An Investigation of the Antitumor Activity of Sesbania drummondii (Fabaceae). *J. Nat. Prod.* (in press).

POWELL, R. G. AND C. R. SMITH, JR. Antitumor Agents from Higher Plants. In Recent Advances in Phytochemistry, Edited by T. Swain and R. Kleiman, Plenum Press, New York, NY, 1980, Vol. 14, Chapter 2, pp. 23-52.

POWELL, R. G., C. R. SMITH, JR., D. WEISLEDER, D. A. MUTHARD, AND J. CLARDY. Sesbanine, A Novel Cytotoxic Alkaloid from Sesbania drummondii. J. Am. Chem. Soc. 101 (1979):2784-2785.

SMITH, C. R., K. L. MIKOLAJCZAK, AND R. G. POWELL. Harringtonine and Related Cephalotaxine Esters. In Design and Synthesis of Anticancer Agents Based on Natural Models, Edited by J. M. Cassady and J. D. Douros, Academic Press, 1980, Chapter 11, pp. 391-416.

WEISLEDER, D., R. G. POWELL, AND C. R. SMITH, JR. Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Cephalotaxus Alkaloids. Org. Magn. Reson. 13 (1980):114-115.

Other Reports:

POWELL, R. G., D. WEISLEDER, AND C. R. SMITH, JR. Novel Maytansinoid Tumor Inhibitors from Trewia nudiflora. American Chemical Society Meeting, Las Vegas, NV, August 24-29, 1980.

WEISLEDER, D. ^{13}C NMR Spectroscopy of Natural Products. Society for Applied Spectroscopy Meeting, Chicago, IL, October 27-31, 1980.

WEISLEDER, D. ^{13}C NMR of Epoxy Fatty Acids. ISF-AOCS Congress, New York, NY, April 27-May 1, 1980.

3. Major Fatty Acids from Indian Seed Oils and Their Possible Industrial Use (P.L. 480 Grant - Aligarh Muslim University)

- a. Specific Objective: Discover and isolate new, naturally occurring fatty acids or other lipids from plant sources. Synthesize new fatty acid derivatives which incorporate halogens, nitrogen, oxygen sulfur, or other heteroelements. Emphasize multifunctional derivatives and determine their reactivity. Discover compounds with morphogenic or behavior-modifying activity against selected economically important insects.

Progress: A series of novel enolacetates have been prepared from various keto acids. Likewise, a series of new thiazoles and oxazoles were prepared from α -halo acids and α -halo keto acids. The reaction of iodoazide (IN_3) with β - and γ -hydroxy acid esters (ricinoleate and isoricinoleate) have been investigated. Products from ricinoleate are those resulting from simple double-bond addition, but those from isoricinoleate involve formation of 1,4-epoxides. The seed oil of Vernonia roxburghii contains 74% epoxy acid of which 17% is the previously unknown *cis*-3,4-epoxy-*cis*-11-octadecenoic acid; the remainder is the more familiar vernolic acid. Wrightia coccinia seed oil was found to be the richest source of isoricinoleic acid yet found. Sterculia foetida seed oil and undecanoic acid iodoazide were found to be active in controlling a flour beetle, Tribolium castaneum.

Publications:

AHMAD, F., NASIRALLAH, S. F. SIDDIQI, AND S. M. OSMAN. Addition of N,N-dibromobenzene Sulfonamide to a Terminal Olefinic Fatty Acid. *Fette Seifen Anstrichm.* (in press).

ALI, M. L., M. S. AHMAD, F. AHMAD, AND S. M. OSMAN. *Ochna squarrosa* Seed Oil: A Richest Source of Tripalmitin. *Chem. Ind.* (in press).

HUSAIN, S., M. U. AHMAD, AND S. M. OSMAN. A New Hydroxy Fatty Acid from Seed Oil of *Baliospermum axillare*. *Phytochemistry* 19 (1980):75-77.

SHERWANI, M. R. K., S. Q. HASAN, I. AHMAD, F. AHMAD, AND S. M. OSMAN. Reinvestigation of the Seed Oil of *Dodonea viscosa* (Sapindaceae). *Chem. Ind.* (1979):523-524.

SIDDIQUI, S. F., F. AHMAD, M. S. SIDDIQI, AND S. M. OSMAN. *Wrightia coccinea* Seed Oil: A Rich Source of Isoricinoleic Acid. *Chem. Ind.* (in press).

C. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO
IMPROVE CROP PRODUCTION

1. Plant Cell and Tissue Culture for the Bioproduction of Valuable Chemicals
(N. E. Delfel)

- a. Specific Objective: Develop new test systems for the study of epigenetic change and cell selection.

Progress: Cultures established from seeds of *Daucus carota*, *Brassica rapa*, and *Chenopodium quinoa* are growing well. New cultures have also been reestablished from *Cephalotaxus harringtonia* needles. Further studies await delivery of suitable microscope equipment.

- b. Specific Objective: Determine seasonal variation in alkaloid level in *C. harringtonia* field-grown trees.

Progress: Samples taken in April from trees in Pennsylvania and Maryland prior to the seasonal flush of new growth gave the same result as in the previous year, high levels of cephalotaxine-degradation products and no detectable levels of antitumor cephalotaxine esters. A greenhouse-grown tree in Maryland showed normal amounts of the esters.

- c. Specific Objective: Determine reason for high alkaloid-ester levels in laboratory-grown *C. harringtonia* tree.

Progress: Six newly acquired small trees grown in growth chambers in pots under 16 hours of artificial light show the same 20- to

100-fold higher alkaloid ester levels compared to field-grown trees as found in a previous study with a single laboratory-grown tree. High ester content, therefore, is more likely due to cultural differences than to intraspecies genetic variations. That alkaloid accumulation in the pot soil is not a factor was shown by GC-MS analysis. Alkaloid storage capacity in field-grown trees is not a limiting factor; thus, if properly stimulated, such trees could be much more productive.

- d. Specific Objective: Reestablish alkaloid biosynthesis in C. harringtonia callus cultures.

Progress: Experiments testing the effect of light and temperature on alkaloid biosynthesis were ruined by equipment failure, as was an experiment involving cell suspension culture. Calluses tested every 2 weeks up to 20 weeks of age failed to show alkaloid production. New cultures are being initiated on various complex media, recapitulating original conditions under which biosynthesis was found. Feeding 3-month-old callus cultures with precursors or possible phenolic intermediates in alkaloid biosynthesis stimulated production of a compound having the GLC retention time of cephalotaxine (to be identified by GC-MS).

- e. Specific Objective: Localize or quantitate alkaloids in cultures or whole plants via immunochemical techniques.

Progress: Atropine heisuccinate attached to bovine serum albumin failed to produce detectable antibodies against atropine in either rabbit or mouse. Alternative methods of coupling that avoid possible ester lability are being evaluated with this alkaloid and similar haptens.

Publications:

DELFEL, N. E. Alkaloid Distribution and Catabolism in Cephalotaxus harringtonia. Phytochemistry 19 (1980):403-408.

DELFEL, N. E. Effect of Nutritional Factors on Alkaloid Metabolism in Cephalotaxus harringtonia Tissue Cultures. Planta Med. 39 (1980):168-179.

Other Reports:

DELFEL, N. E. AND L. J. SMITH. Effect of Medium Composition on Callus Growth of Cephalotaxus harringtonia. Symposium on Propagation of Higher Plants Through Tissue Culture: Emerging Technologies and Strategies. University of Tennessee, Knoxville, October 12-15, 1980.

2. Photosynthetic Pigments and Primary Photoacts for Increasing Efficiency and Yield of Crops (J. A. Rothfus)

- a. Specific Objective: Correlate the spectral absorption of chloroplast pigments with the action spectra of carotenoid sensitized photosynthesis in green and yellow soybean isolines.

Progress: Studies of photosynthetic rates at high light intensity reveal an interesting pattern of very high photosynthetic rates for the pigment deficient genotypes on a pigment basis. The pigment-deficient H_{y9y9} and H_{y3y3} have photosynthetic rates from 9 to 3 times higher than H_{L1} and H_{y7y8} based on chl A, chl B, and total pigments. This phenomenon appears to be indicative of chloroplast structure containing a normal amount of photosystem I and II particles but lacking antenna pigment.

Fluorescence emission spectra of leaf sections of the four genotypes were studied at 77°K. These spectra reveal chlorophyll emission at F 685, F 695, and F 740. The peak height of F 740 relative to that of F 685 and F 695 has been ~~for~~ related to the pigment content of the several genotypes. (i.e. $\frac{F\ 740}{F\ 695}$ α [pigment]).

- b. Specific Objective: Investigate photorespiratory losses in green and yellow soybean isolines through stable isotopes of oxygen and of carbon in carbon dioxide (cooperative with SEA-AR, University of Illinois).

Progress: Photosynthetic and photorespiratory rates for four genotypes of soybean were followed by measurement of CO₂ evolution using mass spectrometric analysis. Photorespiratory rates were found to correlate with photosynthetic rates (P.R. α P.S.). On the other hand, the several genotypes showed large variations of P.S. rates based on leaf area and plant age.

- c. Specific Objective: Investigate dependence of energy transfer on structural arrangements of chlorophylls and carotenoids in model systems and in chlorophyll-caroteno-proteins isolated from chloroplasts.

Progress: Chlorophylls, pheophytin and carotenoid pigments have been adsorbed on hydrophobic and hydrophilic substrates formed from sugar, glass, treated glass and treated silica. Surface areas of substrates and conditions for adsorption have been determined. Alkyl quaternary ammonium chlorides with chains of 16 to 18 carbons have been found to form satisfactory hydrophobic substrates when chemisorbed on glass and to satisfactorily cap C-18 treated silica for use as an adsopriton substrate.

Energy transfer from carotenoid to chlorophyll appears to occur in model systems formed by adsorbing extracts from Nitzschia closterium on a hydrophobic substrate. Energy transfer from fucoxanthin to chlorophyll adsorbed on this same substrate is being investigated.

- d. Specific Objective: Monitor primary light reactions of the oxygen evolving reaction center and the effects of magnesium ion on the arrangement of this reaction center's antenna pigments.

Progress: This is a continuation of work reported last year. New conclusions are: (1) The kinetics processes involved in double advancement in S-states for oxygen evolution are quite distinct from those involved in double turnovers in charge separation, the former being in a range of microseconds while the latter is in a range of tens of nanoseconds. (2) Based on experiments with chloroplasts incubated with ferricyanide, a unique solution to the oxygen S-state distribution in the dark suggested by Thibault (C.R. Acad. Sci. Paris 287 [1978] 725-728) can be rejected.

It is well known that mono- and divalent cations have large effects on photoreactions taking place in isolated thylakoids. Hypotheses for these effects have included regulation of energy distribution between photosystems I and II, changes in radiationless excitation, and control of the activity of photosystem II (PS II) reaction centers. Based on the flash yield for O₂ evolution, it is concluded that the activity of PS II reaction centers is not affected by cation depletion. The effect of divalent cations on O₂ evolution was measured, using both concentration and rate electrodes. Earlier Bose and Arntzen (Arch. Biochem. Biophys. 185:567, 1978) observed an inhibition in O₂ flash yield by cation depletion, and this inhibition was relieved by addition of MgCl₂. They concluded that Mg²⁺ induced an activation of PS II reaction centers. It was observed that the apparent inhibition in O₂ flash yield is actually due to slow settling of cation-depleted thylakoids on the electrode surface, which results in lower O₂ flash yield. Addition of MgCl₂ enhances O₂ flash yield by greatly increasing thylakoid settling rates but not by activating PS II reaction centers. Oxygen yield studies with chlorella and chloroplast using short pulses of light from a Xenon flash lamp and a pulse cly-laser have been repeated.

- e. Specific Objectives: Compare changes in contents of chlorophyll and carotenoid pigments during greening and maturation of various yellow and green isolines of soybeans.

Progress: Eleven soybean genotypes (normal and pigment-deficient mutants) of families Clark and Harosoy were analyzed for chlorophylls and carotenoids during a growing season. Changes in the major chloroplast pigments were studied as a function of leaf age, plant age, and genotype. Those genotypes that exhibit a lag in production of pigments during leaf development showed a similar lag in overall

plant pigment development. Strong correlation exists between pigment-deficient genotypes and immature normal plants.

- f. Specific Objective: Study the changes in pigment levels and interaction between carotenoids and chlorophylls during greening in (1) chlorophyll deficient genotypes of soybeans and (2) etiolated red kidney beans.

Progress: A study of the greening process using etiolated red kidney beans led to the development of a HPLC system for the determination of protochlorophyllide, chlorophyllide, and intermediates in the production of chlorophyll *a*. New forms of chlorophyll *a*, which are important in the early stages of greening, were discovered and partially characterized. Kinetic studies of protochlorophyllide photoconversion were made using the HPLC method of analysis.

- g. Specific Objective: Study relationship of chloroplast pigments to photosynthetic efficiency in corn genotypes (cooperative work with Dr. Doyle Peters, SEA-AR, University of Illinois).

Progress: Analysis of several corn mutants, which range from low to high yielding varieties, indicated a positive correlation between chloroplast pigment levels and productivity.

- h. Specific Objective: Characterize the light-harvesting carotenoid chlorophyll *c*-chlorophyll *a*-protein of *Phaeodacylum tricornutum* with respect to size, lipid content, and nature of bonding between protein subunits.

Progress: The presence of hydrophobic environments within the isolated complex was demonstrated through its affinity for sodium lauroyl sarcosinate (SLS*c*) relative to other proteins in the cell. Since sarcosine (N-methyl glycine) is not present in *P. tricornutum*, it served as a tag to determine the binding power of SLS*c* to the complex. A comparison of the ratio of SLS*c* added to disrupted cells to their protein contents, and the ratio of sarcosine to protein in the complex-isolate, showed at least 3-4 times more surfactant binds to the complex (on a protein basis) than to all other protein in the cell.

The elutriation behavior of the complex on Sepharcyl 200 and phenyl sepharose was consistent with its hydrophobic nature. The complex failed to elute when washed with tris buffer containing various salts (up to 3M concentrations) and 20% ethylene glycol but eluted readily from Sepharcyl 200 (but not phenyl sepharose) when washed with tris buffer containing 0.2M NaCl and either 0.1% Triton X-100 or 0.1% SLS*c*. Further, the complex was characterized with respect to size by electron transmission microscopy and to possible lipid content by gas chromatography. The isolated complex was

studied at 10-50K magnification (at 0.5-1% concentrations) with and without uranyl acetate stain and found to have sizes ranging from 100-500 Å (with an estimated, average-size of 100-200 Å). Also, the isolated complex was found to contain 10 times more protein than lipid. After hydrolysis and methylation, the lipids were assayed as methyl esters of fatty acids and found to contain 47% C₁₆ fatty acids, 20% C₁₈ fatty acids, and 17% of a C₂₀ fatty acid containing 5 double bonds. Chromatography of the complex-preparation on Sephadryl 200 using tris buffer containing 0.2M NaCl gave a 10% fraction of protein that was void of color. This fraction and the 10% lipid fraction suggests that membrane material may be present in the preparation as a contaminant.

Publications:

ESKINS, K. AND L. HARRIS. High-Performance Liquid Chromatography of Etioplast Pigments in Red Kidney Bean Leaves. Photochem. Photobiol. In press.

ESKINS, K., L. HARRIS, AND R. L. BERNARD. Genetic Control of Chloroplast Pigment Development in Soybeans as a Function of Leaf and Plant Maturity. Plant Physiol. In press.

BANK, D. AND K. ESKINS. Analysis of Normal and Mutant Peanut Varieties. Peanut Sci. In press.

GUGLIELMELLI, L. A., H. J. DUTTON, P. A. JURSINIC, AND H. W. SIEGELMAN. Energy Transfer in a Light-Harvesting Carotenoid-Chlorophyll c-Chlorophyll a-Protein of *Phaeodactylum tricornutum*. Photochemistry and Photobiology; accepted December 1980.

EISSLER, R. L. AND H. J. DUTTON. Energy Transfer from Chlorophyll b to Chlorophyll a in a Hydrophobic Model System. Accepted for publication in Photochemistry and Photobiology.

JURSINIC, P. Investigation of Double Turnovers in Photosystem II Charge Separation and Oxygen Evolution with Excitation Flashes of Different Duration. Biochem. Biophys. Acta (accepted October 10, 1980).

Other Reports:

JURSINIC, P. Different Effects of State I and State II and Divalent Cations on Inhomogeneity of Antenna-Pigment Aggregation. Presented at Fifth International Congress on Photosynthetic Research, September 14 & 15, 1980, Attiki, Greece.

EISSLER, R. L. AND H. J. DUTTON. Chlorophyll a and Chlorophyll b on Hydrophobic Substrates. Presented at 1980 Midwest Meeting of Photosynthesis. Marshall, Indiana, October 27, 1980.

GUGLIELMELLI, L. A. The Light-Harvesting Pigment-Protein Complex of Phaeodactylum tricornutum. Presentation at the Midwest Photosynthesis Meeting, Marshall, Indiana, October 26-28, 1980.

YEAZEL, A. M., K. ESKINS, AND L. HARRIS. The Greening of Etiolated Red Kidney Beans. Presented at Seventy-third Annual Meeting, Illinois State Academy of Science, Lisle, IL, April 18-19, 1980.

ZENKER, S., L. HARRIS, AND K. ESKINS. Light Initiated Formation of Forms of Chlorophyll a in Etiolated Red Kidney Beans. Presented at Annual Meeting, Midwest Photosynthesis Group, Marshall, IN, October 27, 1980.

HARRIS, L. AND K. ESKINS. A Cooperative Undergraduate Research Program in Photosynthesis. Presented at American Chemical Society National Meeting - Division of Chemical Education. April 1981.

ESKINS, K., L. HARRIS, AND R. L. BERNARD. Genetic Control of Chloroplast Pigment Development as a Function of Leaf and Plant Maturity. Presented to Genetic Group, University of Oklahoma, Stillwater, OK. March 3-5, 1980.

ESKINS, K., W. F. KWOLEK, AND L. HARRIS. The Accumulation of Accessory Pigments as a Function of Chlorophyll a. A Comparison of Maturity and Genetic Control. Presented at Annual Meeting, Midwest Plant Physiology. June 15-17, 1980.

D. TECHNOLOGIES FOR FOOD AND FEED USES FOR FIELD CROPS

1. Composition and Properties of Seed Lipids for Foods and Feeds (J. A. Rothfus)

a. Specific Objective: Characterize thermal behavior of unsaturated fatty acids and related compounds.

Progress: The melting and freezing properties of 31 different C₁₈-C₂₂ acids, esters, and alkanes were recorded via differential scanning calorimetry. Polymorphism and solid reorientation characteristics are difficult to predict from results with this still limited collection of compounds, but cis-unsaturated monoenes and dienes predominated among compounds exhibiting multiple endotherms or reorientation exotherms; for example, 18:2^{9c12c} acid is dimorphic while 18:2^{9t,12t} is monomorphic. cis-Trienes and cis-tetraenes, however, were more often monomorphic. Among 11 such polyunsaturated compounds, only two trienes and two tetraenes exhibited prominent reorientation exotherms that might indicate polymorphism. Quenched melts exhibited two types of exothermic transitions; one in which the transition temperature was independent of cooling rate, as might be expected of a true polymorph; another in which transition

temperatures varied with cooling rate, reminiscent of glasses. Enthalpies associated with either type were cooling-rate dependent. Entropies of fusion for methyl esters were generally higher, on a per carbon basis, than those for acids, indicating a higher degree of order in the ester crystals. Among compounds examined thus far, oleic acid showed the greatest change in fusion entropy after esterification.

Studies on single acid triglycerides of 9,10-deuterio-octadecadienoic acids were completed for all four possible geometric isomers without detecting significant isotope effects by DSC.

- b. Specific Objective: Obtain Raman spectra for polymorphic forms of triglycerides.

Progress: Techniques were perfected for preparing four tristearin polymorphs that exhibit distinctly different Raman spectra. In addition to α - and β -forms, DSC characterization identified two of these materials as β' -forms. This is the first spectral evidence that β' -forms seen previously in DSC studies represent significant polymorphs.

- c. Specific Objective: Continue computer modeling of triglyceride structures.

Progress: Incorporation of a minimization subprogram reduced by ca. one half the time required to identify prospective stable molecular arrangements in a geometric model of α -form triarachidin. Calculations are thus complete for all four possible conformations with both synchronous and nonsynchronous oscillation of hydrocarbon chains about their axes. Chain synchrony had little effect on calculated interaction energies and organization in the hexagonal crystal. This is consistent with X-ray analyses that suggest there is considerable freedom for movement of chains within such crystals. Chain oscillation is expected to be a more critical determinant of stable conformations in crystals that involve chain tilt. Molecular positions based on interactions with two adjacent molecules being refined by including interactions with parts of four to six additional surrounding molecules. Such refinement has reduced the number of potentially equivalent stable structures from 23 to 14. Seven are known to have very similar high interaction energies. Interestingly, those most likely from the standpoint of crystal symmetry are not necessarily the most stable in terms of interaction energy.

A plotter program, developed to better visualize chain orientation and allow direct measurements relating to molecular dimensions from X-ray analyses, demonstrated that triglyceride dimensions can be changed to fit β' -long spacings by single bond rotations in either chain 2 or chains 1 and 3.

Publications:

CHANG, S. P. Thermal Behavior of Ordered and Disordered Crystals from Fatty Acids, Esters, and Alkanes. J. Am. Oil Chem. Soc. 57(2) (1980):100A.

PARRISH, F. W., K. D. ROSS, AND T. D. SIMPSON. Formation of β -lactose from α - and β -lactose Octaacetates, and from α -lactose Monohydrate. Carbohydr. Res. 71 (1979):322-326.

SIMPSON, T. D. Crystallography. In Fatty Acids, Edited by E. H. Pryde, American Oil Chemists' Society, Champaign, IL, Chapter 8, pp. 157-172, 1979.

Other Reports:

CHANG, S. P. Thermal Behavior of Ordered and Disordered Crystals from Fatty Acids, Esters, and Alkanes. Presented at the ISF/AOCS World Congress, New York, April 27-May 1, 1980.

2. Soybean Analysis for Improved Quality (R. Kleiman)

- a. Specific Objective: Determine oil and protein contents of soybean samples in order to develop improved varieties.

Progress: About 13,500 samples were received from public soybean breeders throughout the United States and Canada. These samples were examined for their oil and protein content by the infrared reflectance method. Five samples grown in a CSIRO phytotron to determine temperature response of seed growth characteristics of soybeans were analyzed and the results are as follows:

Growth

temperature

range, °C (day/night)	%	%	Fatty acids, %				
			Oil	Protein	16:0	18:0	18:1
18/13	14.0	35.1			11.1	3.2	13.0
24/19	15.8	35.1			11.7	3.2	19.8
27/22	18.1	33.8			11.6	3.6	22.3
30/25	18.0	33.9			12.0	3.6	29.2
33/28	19.2	42.2			12.0	3.1	38.7
							40.3
							5.0

Growing conditions thus have a significant effect on the oil content and on the composition of the oil. The protein content is not

affected as much, and analysis of the protein did not reveal significant differences in amino acid composition.

- b. Specific Objective: Provide fatty acid compositional analysis of selected soybean samples in order to lower the linolenic acid content through plant breeding.

Progress: Fatty acid composition was determined on 5,000 soybean samples. Included in these gas chromatographic analyses were germplasm collection samples from the northern and southern collections. Several samples with linolenic acid content less than 5% were found. The lowest of these was 4.2%. This sample was also unusual in that the 18:2 component was higher than usual (58%). Most samples with decreased 18:3 content are higher in 18:1. Analysis of chemical mutation samples from J. Wilcox, SEA-AR, Purdue University, yielded no low linolenic acid samples.

- c. Specific Objective: Develop rapid analytical procedures for soy protein in order to raise the methionine and cystine content through breeding.

Progress: Ion exchange chromatography equipment has been combined with a modified elution procedure to produce a 45-minute chromatographic analysis procedure for methionine and cystine of soybeans. This procedure was applied to over 100 samples.

Publications:

CAVINS, J. F. Nitrogen Analysis of Whole Seeds. *J. Am. Oil Chem. Soc.* 57(8) (1980):665A-656A.

PLATTNER, R. D., R. O. BUTTERFIELD, AND T. D. SIMPSON. The Microcomputer as an Intelligent Interface Between Laboratory Instruments and Data Systems: Automating Data Collection from a Grain Analyzer. *Chem. Biomed. Environ. Instrum.* 10(3) (1980):331-338.

E. RECLAMATION AND REVEGETATION OF
LAND AREAS DISTURBED BY MAN

1. Trace Element Uptake and Distribution in Agricultural Crops Grown on Disturbed Lands (K. D. Carlson)

- a. Specific Objective: Evaluate effect of sewage sludge application to stripmined land on the growth of nonfood crops, notably crambe and kenaf.

Progress: Crambe and kenaf were grown a second year on stripmined plots established in 1979. The same treatments were used: control (C), commercial fertilizer (CF), 50 ton/acre dewatered sludge (S₅₀)

and 100 ton/acre sludge (S_{100}). Excellent uniform stands of both crops were obtained under all treatments. Barnyard grass competition in S_{50} and S_{100} plots affected the performance of both crops, particularly crambe.

Kenaf yields were significantly lower on S_{100} plots than on others. Crambe seed yields for 1980 have not been determined. For the 1979 year, seed yields were in the order CF> S_{50} >C> S_{100} . A third planting year is planned for 1981.

- b. Specific Objective: Determine uptake by these nonfood crops of heavy metals that may be contained in sewage sludge.

Progress: All samples from the 1979 crop year are now ready for heavy metal analyses. Soil samples from the plots prior to 1979 amendments have been analyzed for total Zn, Cd, Cu, Ni, exchangeable Mn, K, Na, Ca, Mg; available P; organic C; conductivity and pH.

Reports:

CUNNINGHAM, R. L. AND K. D. CARLSON. Crambe and Kenaf Grown on Stripmine Land Amended with Sewage Sludge. Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, IL, February 7, 1980.

CUNNINGHAM, R. L. AND K. D. CARLSON. Crambe and Kenaf Grown on Stripmine Land with Sewage Sludge. Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, IL, May 9, 1980.

CUNNINGHAM, R. L. AND K. D. CARLSON. "Biomass and Energy Research at the Northern Agricultural Energy Center" and "Crambe and Kenaf Grown on Stripmine Land Amended with Sewage Sludge as Related to Biomass Production." Biomass Coordinating Meeting, Metropolitan Sanitary District of Greater Chicago, Wee-Ma-Tuk Country Club, Fulton County, IL, July 1, 1980.

CUNNINGHAM, R. L. AND K. D. CARLSON. Progress Report on Crambe and Kenaf Grown on Stripmine Land Amended with Sewage Sludge. Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, IL, August 22, 1980.

CUNNINGHAM, R. L. AND K. D. CARLSON. Progress Report on Crambe and Kenaf Grown on Stripmine Land Amended with Sewage Sludge. Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, IL, November 14, 1980.

F. UTILIZE, MANAGE, AND CONSERVE SOIL FERTILITY FOR
INCREASED PRODUCTION AND NUTRITIONAL QUALITY OF
PLANTS AND ANIMALS

1. Improve and Implement the Determination of Isotopic Nitrogen in Soil Samples (R. Kleiman)
 - a. Specific Objective: Cooperate with SEA soil scientists in determining amount of ^{15}N developed through nitrogen X tillage experiments.

Progress: Mass spectrometer conditions were optimized and working standards were developed. Eleven hundred samples were received from Oregon, Kentucky, and Nebraska. These samples were analyzed for ^{15}N content.
 - b. Specific Objective: Develop automatic procedure for conversion of NH_4Cl to N_2 and introduction into the mass spectrometer for analysis to atom % N.

Progress: A commercial automatic sampler was greatly modified so its mechanical functions could be controlled manually or by the on-board computer of the mass spectrometer. Additional equipment was constructed to introduce sodium hypobromite to the NH_4Cl sample to control vacuum and introduce N_2 into the mass spectrometer. These functions are accomplished under computer control. Work is underway to optimize total automation.

G. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Cattle Feeding Tests of Crambe Meal as a Protein Concentrate (Cooperative Agreement - Purdue University)
 - a. Specific Objective: Obtain FDA clearance for using crambe meal in beef cattle rations.

Progress: We are currently awaiting formal approval by FDA, which is expected to be announced in the Federal Register in the next 3 months. No work is planned or in progress.
2. Inheritance of Glucosinolates in Crucifer Vegetables (Cooperative Agreement - University of Wisconsin)
 - a. Specific Objective: Study genetics of glucosinolates in the genera Brassica and Raphanus.

Progress: Work was initiated in June 1980 to develop rapid cycling stocks of seven species from the above genera. B. campestris can be grown from seed to flowering in 16 days.

3. Natural Toxicants in Horticultural Crops and Cruciferous Feeds (H. L. Tookey)

- a. Specific Objective: Determine biological activity of selected glucosinolate products that occur in significant amounts in crucifer vegetables.

Progress: The effects of (R)-goitrin on the liver are currently under study using rats as test animals.

- b. Specific Objective: Discover convenient sources of natural toxicants needed for future testing.

Progress: A convenient source of 4-methylthiobutyl glucosinolate (which occurs in rutabaga) has been found in seed of Sisymbrium sp. A novel glucosinolate has been discovered in Berteroa incana seed and characterized as dextrorotatory 2-hydroxy-4-pentenyl glucosinolate.

- c. Specific Objective: Determine effects of edaphic conditions on the glucosinolate content of cabbages.

Progress: Cabbages grown under several different levels of moisture and fertilizer did not show significant differences in glucosinolate content. More extensive work would be needed to yield a definitive answer.

- d. Specific Objective: Provide data basis for evaluation of newly developed cultivars in regard to their glucosinolate content.

Progress: Glucosinolates of 41 cultivars of turnip and rutabaga range from 216 to 514 μ mole per 100 g peeled root. Progoitrin, a major component of most cultivars, is conspicuously absent from turnip cultivars.

- e. Specific Objective: Investigate germplasm variability in crucifer vegetables of Oriental origin.

Progress: Typical vegetables from Japan and Taiwan have been received from the University of Wisconsin for analysis. These include Brassica campestris subspecies chinensis rapa, nipponsinica, and Raphanus sp.

- f. Specific Objective: Investigate scope and specificity of components that yield epithionitriles from glucosinolates.

Progress: Work has been initiated on the specificity of enzyme and other factors from species other than Crambe.

- g. Specific Objective: Cooperate with other laboratories in evaluating livestock feeds that may contain natural toxicants.

Progress: Chicken diets containing Limnanthes meal were analyzed for cooperators at Oregon State University. The diets contained about 0.5% glucosinolate, primarily methoxybenzyl. Lathyrus sylvestrus (flat pea) forage has been analyzed for cooperators at Soil Conservation Service NE-TSC, Broomall, Pennsylvania. A peak value of 2.2 g/100 g (d.b.) of the toxicant 2,4-diaminobutyric acid appeared in June cuttings.

- h. Specific Objective: Develop and validate methods for the assay of toxicants of carrot roots.

Progress: Falcarinol analyses are sensitive to 10 p.p.m. with good precision and recoveries of 90%. Methods for falcarindiol and myristicin are precise but require additional validation for recovery.

- i. Specific Objective: Determine levels of toxicants in carrot roots.

Progress: In four cultivars, falcarinol ranged from 6 to 31 p.p.m.; falcarindiol, from 73 to 285 p.p.m.

Publications:

DAXENBICHLER, M. E., G. F. SPENCER, and W. P. SCHROEDER. 3-Hydroxypropylglucosinolate, A New Glucosinolate in Seeds of Erysimum hieracifolium and Malcolmia maritima. *Phytochemistry* 19 (1980):813-815.

DAXENBICHLER, M. E., C. H. VanETTEN, and P. H. WILLIAMS. Glucosinolate Products in Commercial Sauerkraut. *J. Agric. Food Chem.* 28 (1980):809-811.

GOULD, D. H., M. R. GUMBMANN, and M. E. DAXENBICHLER. Pathological Changes in Rats Fed the *Crambe* Meal-Glucosinolate Hydrolytic Products (2S)-1-Cyano-2-hydroxy-3,4-epthiobutanes (erythro and threo) for 90 Days. *Food Cosmet. Toxicol.* 18 (1980):619-625.

NISHIE, K., and M. E. DAXENBICHLER. Toxicology of Glucosinolates, Related Compounds (Nitriles, R-Goitrin, Isothiocyanates) and Vitamin U Found in Cruciferae. *Food Cosmet. Toxicol.* 18 (1980):159-172.

SPENCER, G. F., and M. E. DAXENBICHLER. Gas Chromatography-Mass Spectrometry of Nitriles, Isothiocyanates, and Oxazolidinethiones Derived from Cruciferous Glucosinolates. *J. Sci. Food. Agric.* 31 (1980):359-367.

TOOKEY, H. L., M. E. DAXENBICHLER, C. H. VanETTEN, W. F. KWOLEK, and P. H. WILLIAMS. Cabbage Glucosinolates: Correspondence of Patterns in Seeds and Leafy Heads. *J. Am. Soc. Hort. Sci.* 105 (1980):714-717.

VanETTEN, C. H., M. E. DAXENBICHLER, H. L. TOOKEY, W. F. KWOLEK, P. H. WILLIAMS, and O. C. YODER. Glucosinolates: Potential Toxicants in Cabbage Cultivars. *J. Am. Soc. Hort. Sci.* 105 (1980):710-714.

WEISLEDER, D., and E. LILLEHOJ. Carbon-13 Nuclear Magnetic Resonance Assignments and Biosynthesis of Ochratoxin A. *Tetrahedron Lett.* 21 (1980):993-996.

WILLIAMS, P. H., and M. E. DAXENBICHLER. Glucosinolates in Chinese Cabbage. International Symposium on Chinese Cabbage Proceedings, Tsukuba, Japan, March 31-April 5, 1980. In press.

Other Reports:

ENGLAND, R. E., and S. G. YATES. Isolation of Polyacetylenes from Carrot. Southern Illinois University Chemistry Conference, Carbondale, IL, October 17-18, 1980.

OILSEED CROPS LABORATORY

T. L. Mounts, Chief

Research Leaders: E. A. Emken, E. N. Frankel, E. H. Pryde, and
W. J. Wolf

A. TECHNOLOGIES FOR FOOD AND FEED USES FOR FIELD CROPS

1. Effects of Vegetable and Animal Trypsin Inhibitors in Long-Term Animal Feeding Studies (Cooperative Agreement - University of Minnesota)

- a. Specific Objective: Continue studies on biochemical affects of animal and vegetable trypsin inhibitors (TI) on rat pancreas.

Progress: The first series of feedings of 26 diets that were initiated on September 12, 1979 (AES, University of Minnesota), and October 26, 1979 (Western Regional Research Center, SEA-AR), have progressed very well in all phases. Survival rate of rats on all diets is very good. Analyses of the moisture and nitrogen contents and microbial profiles of the six dietary soy products and casein indicate good stability after storage at 2-4°C and relative humidity of 60-65%. Samples at 0, 3, 6, and 12 months storage were analyzed. The dietary products and diets prepared from them were Salmonella-free, and had low plate counts. No toxin-producing microorganisms were found.

Quantitation of the histopathological changes in the excised pancreas of rats fed diets containing varying levels of TI in the presence of 10, 20, and 30% dietary protein from rats sacrificed at 6 and 9 months indicate no significant changes compared with rats fed casein control diets (except for enlargement of the pancreas with diets containing high levels of TI activity). The dispersed endocrine system of the intestinal mucosa also revealed no abnormalities.

Complete enzyme analyses of the excised pancreas and fecal matter of the sacrificed rats correlate well with in vivo protein synthesis in the pancreas and secretion of pancreatic juice enzymes. The in vivo experiments involved the measurement of the hydrolytic product excreted into the urine when a synthetic substrate, specific for the proteolytic enzymes of the pancreas, was fed to the same group of rats for 5 days prior to sacrifice at 6, 9, and 12 months. Analysis of comparative data obtained by in vivo and in vitro methods demonstrated that high casein diets increased protein synthesis and secretion of pancreatic enzymes, cause pancreatic hypertrophy and inhibit growth. These results correlated with

similar effects observed with diets containing high levels of soybean TI activity.

- b. Specific Objective: Prepare research protocols for all phases of the long-term feeding studies.

Progress: To comply with requirements of the FDA for Good Laboratory Practices and Quality Assurance Units, protocols were written for the life-time rat feeding tests. Managers, study directors, and Quality Assurance Unit personnel have been selected. Details are described in research protocols.

2. Improving Food Quality of Soy Oil Products and Their Stability to Heat- and Light-Catalyzed Oxidation (E. N. Frankel)

- a. Specific Objective: Develop methodology and isolation techniques to monitor changes in heated oils caused by thermal oxidation.

Progress: A method was previously reported for the analysis of materials with the same retention as cyclic monomers in soybean oils and hydrogenated soybean oils used repeatedly to fry potato slices at 195°C. Peaks corresponding to cyclic acids were now further identified by gas chromatography-mass spectrometry (GC-MS) as due to ortho-disubstituted propyl and butyl branched cyclic C-18 acids. In addition to the cyclic monomers, the gas chromatograms of heated oils showed significant amounts of polar oxidation materials that have not been identified.

Pure diunsaturated C₁₈ cyclic fatty acid methyl esters (CFAME) of known structure were synthesized to assess their biological activity in heat-abused oils and to serve as reference models in characterizing heated fats. The Wittig reaction was used to prepare: (1) methyl 9-(6-propyl-3-cyclohexenyl)-8-nonenoate, (2) methyl 11-(6-methyl-3-cyclohexenyl)-10-undecenoate, and (3) methyl 12-(3-cyclohexenyl)-1-dodecenoate. The appropriate methyl ω -bromo esters and their triphenylphosphonium bromides were made and converted to their reactive ylids. Substituted and unsubstituted 3-cyclohexenals were reacted with the ylids. The desired CFAME products were isolated in crude yields of 30-83% as liquids and fractionally distilled. These model CFAME and their saturated derivatives were characterized by spectral methods.

- b. Specific Objective: Study the effect of photosensitized oxidation on deterioration of vegetable oils.

Progress: Secondary oxidation of hydroperoxides produces a complex mixture of volatile and nonvolatile compounds that may contribute either directly or as precursors to flavor deterioration in unsaturated fats. To clarify the sources of undesirable flavors, pure hydroperoxides from autoxidized and photosensitized oxidized

fatty esters were thermally decomposed in the injector port of a GC-MS system. Major volatile products were identified from the hydroperoxides of methyl oleate, linoleate, and linolenate. Although the hydroperoxides from autoxidized esters are isomerically different in the position and concentration than those from photosensitized oxidized esters, the same major volatile products were formed but in different relative amounts. Distinguishing volatiles were, however, produced from each type of hydroperoxide.

Photosensitized oleate hydroperoxides produced much more octane, 2-decenal, 2-undecenal, 1-octanol, methyl heptanoate and octanoate, and much less octanal and methyl 10-oxodecanoate than the autoxidized oleate hydroperoxides. The autoxidized linoleate hydroperoxides produced much more pentane, 2-pentylfuran, 2,4-decadienal, and methyl octanoate and much less methyl 10-oxo-8-deenoate and 2-heptenal than the photooxidized linoleate hydroperoxides. The autoxidation linolenate hydroperoxides produced more decatrienal, methyl octanoate, and ethane and less methyl 10-oxo-8-deenoate and 2-butenal than the photooxidized linolenate hydroperoxides. Secondary reactions of hydroperoxides are postulated, and the cyclic peroxide hydroperoxides from methyl linoleate (photosensitized oxidized) and methyl linolenate (both autoxidized and photosensitized oxidized) are suggested as important precursors of volatiles.

A previous study of nonvolatile autoxidation products by high-pressure liquid chromatography (HPLC) of methyl oleate and linoleate was extended to methyl linolenate. Autoxidized methyl linolenate was fractionated by HPLC either after reduction to allylic alcohols on a reverse phase system or directly on a micro silica column. Isolated oxidation products were characterized by thin-layer and gas-liquid chromatography, ultraviolet, infrared, nuclear magnetic resonance, and mass spectrometry. Nonvolatile products from linolenate autoxidation mixtures (containing 3.5 to 8.4% monohydroperoxides) included 9-hydroperoxy-10,12-peroxy-trans,cis/trans,trans-13,15-octadecadienoate and 16-hydroperoxy-13,15-peroxy-cis,trans/trans,trans-9,11-octadecadienoate (3.8-7.7%), epoxy unsaturated compounds (0.2-0.3%), epoxy-hydroxydienes (<0.1%), and dihydroperoxides with conjugated diene-triene and conjugated triene systems (0.9-2.9%). Cyclization of the 12- and 13-hydroperoxides of linolenate would account for their lower relative concentration than the 9- and 16-hydroperoxides. Di-hydroperoxides may be derived from the 9- and 16-linolenate hydroperoxides. Cyclic peroxides and dihydroperoxides are suggested as important flavor precursors in oxidized fats.

- c. Specific Objective: Study the oxidation of isomeric dienes to determine how flavor deterioration occurs in hydrogenated soybean oil.

Progress: Foreign markets for soybean oil have been limited by flavor problems, especially in Southern European countries where consumers object to the unique odor generated by cooking with hydrogenated soybean oil. To investigate the precursors of this flavor and odor deterioration, isolinoleates were prepared with double bonds in the 9,15- and 12,15-positions. These isomeric dienes formed by hydrogenation of linolenate in soybean oil were autoxidized, and the hydroperoxides were identified by gas chromatography-mass spectrometry. Eight isomeric hydroperoxides with one allylic and one isolated double bond were identified from oxidized 9,15-diene, and two conjugated hydroperoxides from 12,15-diene. The 15 double bond in the isolinoleates was preferentially oxidized to form 16- and 17-hydroperoxides in significantly higher proportion than the other isomers. Since hydroperoxides are the main precursors of volatile off-flavors, this work supports the long-held belief that flavor deterioration in soybean oil and hydrogenated soybean oil is associated with the oxidation of the 15 double bond in linolenate and in its derived isomeric dienes. Therefore, a more stable oil could be produced if we could remove more selectively the terminal unsaturation in soybean oil.

- d. Specific Objective: Study immobilized metal complex catalysts for the selective hydrogenation of soybean oil.

Progress: A silica-bonded complex was prepared by reacting polyphenylsiloxane with silylated Chromosorb WAW and then with Cr(CO)₆. This complex catalyzed stereoselective hydrogenation of sorbate to cis-3-hexenoate. Soybean methyl esters were hydrogenated at 210°C in cyclohexane to form products high in cis unsaturation. The recovered catalyst could be recycled once with methyl sorbate. Infrared analysis showed decreased Cr(CO)₃ in the recovered catalysts, and the hydrogenation products contained inactive Cr.

A polymeric palladium catalyst was active for hydrogenation of soybean oil and soybean methyl esters in a fixed bed continuous reactor (138°C, 400 psi H₂). However, the linolenate selectivity was less than that of a commercial supported palladium catalyst. Attempts to hydrogenate soybean esters with cis bond producing chromium carbonyl catalysts in the same equipment were unsuccessful because of packing in the catalyst bed which prevented flow.

- e. Specific Objective: Study photo-catalytic and -inhibiting compounds in crude soybean oil.

Progress: Unsaponifiable constituents of crude soybean oils were fractionated to study their effect on photosensitized oxidation. Because of the instability of several components to both alkali and heat, a nondestructive method was developed for concentrating unsaponifiables by low temperature crystallization of the oil in 99% acetone (-47°C). The main, more saturated triglycerides

crystallized out, while the unsaponifiables stayed in the filtrate. The remaining triglycerides were separated by silicic acid chromatography (10% Et₂O/hexane). The unsaponifiables were fractionated by HPLC and preparative TLC. Components tentatively identified by TLC were β -carotene, α -tocopherol, γ -tocopherol, δ -tocopherol, sterols, and lutein. Tocopherols and chlorophyll were detected by fluorescence spectrophotometry. Saponification of a concentrate of unsaponifiables and triglyceride from the crystallization filtrate yielded several decomposition products not found by the milder method of separation.

Publications:

- AWL, R. A., E. N. FRANKEL, J. P. FRIEDRICH, AND C. L. SWANSON. Tricarbonylchromium-complexed Phenylsiloxane Polymers as Stereoselective Hydrogenation Catalysts: Preparation and Properties. *J. Polym. Sci., Polym. Chem. Ed.* 18 (1980):2663-2676.
- FRANKEL, E. N. Homogeneous Catalytic Hydrogenation of Unsaturated Compounds with Chromium Carbonyl Catalysts. In *Catalysis in Organic Syntheses*, Edited by W. H. Jones, Academic Press, New York, pp. 185-193. 1980.
- FRANKEL, E. N. Lipid Oxidation. *Prog. Lipid Res.* 19 (1980):1-22.
- FRANKEL, E. N. Soybean Oil Flavor Stability. In *Handbook of Soy Oil Processing and Utilization*, Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, American Soybean Association, St. Louis, MO, and American Oil Chemists' Society, Champaign, IL, pp. 229-244. 1980.
- FRANKEL, E. N. Analytical Methods Used in the Study of Autoxidation Processes. In *Autoxidation in Food and Biological Systems*, Edited by M. G. Simic and M. Karel, Plenum Press, New York, pp. 141-170. 1980.
- FRANKEL, E. N. Autoxidation. In *Survey on Lipid Science and Technology 1980*, Reports from the Joint ISF/AOCS Congress, April 27-May 1, 1980, New York, Edited by R. Marcuse, International Society for Fat Research, Lipid Forum, Goteborg, Sweden, pp. 96-100. 1980.
- FRANKEL, E. N., E. J. DUFEK, AND W. E. NEFF. Analysis of Autoxidized Fats by Gas Chromatography-Mass Spectrometry: VI. Methyl 9,15- and 12,15-Octadecadienoate. *Lipids* 15 (1980):661-667.
- FRANKEL, E. N., J. P. FRIEDRICH, T. R. BESSLER, W. F. KWOLEK, AND N. L. HOLY. Hydrogenation of Methyl Sorbate and Soybean Esters with Polymer-Bound Metal Catalysts. *J. Am. Oil Chem. Soc.* 57 (1980):349-354.

NEFF, W. E., AND E. N. FRANKEL. Quantitative Analyses of Hydroxystearate Isomers from Hydroperoxides by High-Pressure Liquid Chromatography of Autoxidized and Photosensitized-Oxidized Fatty Esters. *Lipids* 15 (1980):587-590.

Other Reports:

FRANKEL, E. N. Session Chairman, Autoxidation, Mechanism, and Biological Oxidation, Joint Meeting of the ISF/AOCS World Congress, New York, NY, April 27-May 1, 1980.

FRANKEL, E. N. Stability and Autoxidation. Presented at Conference on Review of Soybean Research, Peoria, IL, May 15-16, 1980.

FRANKEL, E. N. Highlights of Edible Oil Research. Presented at Shortening and Edible Oil Technical Meeting, Peoria, IL, September 17, 1980.

FRANKEL, E. N. Determination of Cyclic Acids to Evaluate Heat Abuse in Vegetable Oils Used for Frying. Presented at Conference of General Collaborators from North Central Agricultural Experiment Stations, Peoria, IL, October 20-21, 1980.

FRANKEL, E. N., J. P. FRIEDRICH, T. R. BESSLER, AND W. F. KWOLEK. Hydrogenation of Methyl Sorbate and Soybean Esters with Polymer-Bound Metal Catalysts. Presented at Joint Meeting of the ISF/AOCS World Congress, New York, NY, April 27-May 1, 1980.

NEFF, W. E., AND E. N. FRANKEL. Quantitative Analyses of Hydroperoxides by High-Pressure Liquid Chromatography. Presented at ISF/AOCS Meeting, New York, NY, April 27-May 1, 1980.

3. Nutritional Quality, Safety, and Flavor Aspects of Soybean Protein Products (J. J. Rackis)

a. Specific Objective: Prepare purified potato trypsin inhibitors (TI's) for long-term rat feeding studies.

Progress: The trypsin inhibitors were first concentrated by processing 24,000 pounds of Kennebec potatoes to remove pulp and starch; the remaining protein water was concentrated by vacuum evaporation to produce a potato juice concentrate (PJC) at Eastern Regional Research Center, SEA-AR. A number of procedures involving heat coagulation, acid precipitation, and ultrafiltration were then investigated at NRRC in order to fractionate and concentrate TI in the PJC. It was found that heat coagulation resulted in a major loss of TI activity. At pH 3.5, noninhibitor-type proteins were precipitated and TI was recovered in a subsequent ultrafiltration separation with only a 10% loss of TI activity.

- b. Specific Objective: Identify and quantitate the amount of cyanide-containing constituents (cyanogens) in soybeans.

Progress: Under mild acid hydrolysis of the meals or citrate buffer extraction of the meals, values of 0.1 to 0.3 µg cyanide (CN⁻)/g were found in whole soybeans, raw defatted and overtoasted soy flour, soy hulls, whey, and residue from soy protein isolate preparation. A value of 1.5 µg/g was obtained on a toasted protein concentrate but no reliable value was obtained for a protein isolate. Carbonyl compounds and SO₃⁻ appear to interfere with analyses under conditions used. Enzymatic hydrolyses with emulsin and a crude lima bean linamarase extract were ineffective although they worked with lima bean linamarin. Eighty percent ethanol and hexane-ethanol extracts of defatted soybean meal were made and fractionated on silicic acid and Sephadex columns, but no cyanogenetic precursors were isolated, and cyanide measured in the extracts was little higher than in the original meal. These results suggest that very low levels of cyanide are present in soybeans, and the higher levels found under charring conditions appear to be artifacts of the assay. Since cyanogenetic precursors appear to be present in too small amounts to be of nutritional consequence, their isolation is not practical.

- c. Specific Objective: Evaluate the role of peroxidases in the generation of objectionable flavors in soy protein products.

Progress: Soybean peroxidases were purified 628-fold with a yield of 4%. From our previous work, we characterized the minor protein impurities as having similar molecular weights but higher net negative charge than our peroxidase. By incorporating a second ion exchange separation into our purification scheme, we improved the enzyme activity over 3-fold. Yields of peroxidase were improved with minor changes in our fractionations and column chromatographies. A molecular weight of 37,200 was estimated for soybean peroxidase via SDS polyacrylamide gel electrophoresis. A sensitive O₂ uptake method with a Clark electrode was developed to assess oxidative role of soybean peroxidases and their ability to oxidize indoleacetic acid (IAA) by molecular oxygen. The extent of O₂ uptake correlated with a sensitive colorimetric assay for IAA oxidation products. Our findings prove that IAA oxidation is dependent on H₂O₂ concentration in the reaction. Therefore, by limiting the amount of H₂O₂ present (e.g., use of catalase), we can control IAA oxidation. With these highly sensitive methods, we can show that linoleic acid hydroperoxide both free and bound to phosphatidylcholine, when incorporated into our model system, will show some O₂ uptake with subsequent oxidation of IAA.

- d. Specific Objective: Correlate sensory evaluation with gas chromatographic (GC) analyses of volatiles in commercial soybean protein samples to provide basis for flavor improvement.

Progress: Forty-three samples of commercially available soy protein flours, concentrates, and isolates (textured and untextured) were evaluated for sensory characteristics as well as volatile constituents. Flavor scores ranged between 3.5-6.3 for flours, 4.4-5.9 for concentrates, 5.1-6.9 for isolates, and 5.3-6.7 for textured flours. Major volatile constituents were residual solvents (ethanol and hexane) and hexanal. Significant correlation coefficients were calculated between overall flavor scores and nine of the 14 GC peaks. Residual solvent had a deleterious effect on soy flavor if present at levels over 300 ppm. Results of this study are being used in determination of specifications for soy in blends with ground beef for military feeding in collaboration with U.S. Army Natick R&D Lab.

- e. Specific Objective: Devise methods to prevent rancidity in diets used in long-term trypsin inhibitor rat feeding studies.

Progress: Casein diets developed oxidative rancidity odors after 2 weeks storage, whereas all diets containing raw or toasted soy flours or isolates exhibited excellent stabilities. With the assistance of the OC sensory panel and BC personnel, it was found that addition of very low levels of the antioxidants TBHQ + BHA (100 ppm with respect to 8% dietary fat) provided excellent oxidative stability at 37°C for up to 6 months storage of the casein diets. Gas chromatographic analyses of volatile compounds showed significant correlations between hexanal content and rancidity as measured by the sensory panel.

- f. Specific Objective: Identify oxidation products of soy phosphatidylcholine (PC) molecular species and evaluate their contribution to the flavor of soy protein products.

Progress: The major molecular species of soy PC have been purified, oxidized to the 10% level, and the resulting hydroperoxides isolated and identified. At this level, there is very little oxidation of 16:0 or 18:1 while 18:2 forms a 9- or 13-monohydroperoxide. In species containing 18:3, the linolenate first forms a monoperoxide at the 9-, 12-, 13-, or 16-position which can then undergo a second oxidation to form 9, 16 conjugated triene or cyclize to form an epidioxide-hydroperoxide.

- g. Specific Objective: Prepare low-TI soy protein isolate for long-term feeding studies.

Progress: A successful commercial pilot plant run was completed to prepare 1,500 pounds of the following protein isolates: (1) raw, soy protein isolate (28-30 mg TI/g sample), (2) low-TI, salt-extracted raw soy protein isolate (4-5 mg TI/g), and (3) salt-extracted soy protein isolate treated with live steam at 290°F for 20 seconds (2-4 mg TI/g). TI values are well within the

specifications for the research protocol to delineate the deleterious effects of TI's on the rat pancreas from that associated with raw, poorly digestible protein. The failure to completely eliminate the low TI activity in product b by steam treatment indicates that the residual TI activity is a measurement of incomplete tryptic hydrolysis rather than the actual presence of protease inhibitors. Strict pH control (pH 5.0 to pH 5.2) is essential to prepare isolates with low TI activity in the presence of 0.1N sodium chloride.

- h. Specific Objective: Determine the kinetics of oxidation and subsequent decomposition of the various molecular species of soy phosphatidylcholine.

Progress: The molecular species of soy phosphatidylethanolamine (PE) have been separated with isotopically labeled 16:0-18:2 PC synthesized by reacting the PE with $^{14}\text{CH}_3\text{I}$. The kinetics of oxidation of this species has been determined.

Publications:

GARDNER, H. W., C. G. CRAWFORD, AND J. T. MACGREGOR. Fatty Epoxides from Degradation of Linoleic Acid Hydroperoxides are not Mutagenic by Ames Test. Proceedings Lipid Oxidation, Vitamin E, Selenium, and Carcinogenesis. In press.

RACKIS, J. J. Significance of Soya Trypsin Inhibitors Activity in Nutrition. J. Am. Oil Chem. Soc. In press.

RACKIS, J. J. Comparison of the Food Value of Immature, Mature, and Germinated Soybeans. Am. Chem. Soc. Symposium Series. In press.

RACKIS, J. J. Flatulence and Its Control Through Soya Processing. J. Am. Oil Chem. Soc. In press.

Other Reports:

CRAWFORD, C. G. Oxidation of Soy Phosphatidylcholine. Presented at Review of Soybean Research, Northern Regional Research Center, Peoria, IL, May 15-16, 1980.

CRAWFORD, C. G. Soy Flavor Studies. Presented to Soybean Utilization Research Advisory Panel, Northern Regional Research Center, Peoria, IL, June 19-20, 1980.

CRAWFORD, C. G. Primary Oxidation Products of Soy Phosphatidylcholine. Presented at the Conference of General Collaborators from North Central Agriculture Experiment Stations, Northern Regional Research Center, Peoria, IL, October 20-21, 1980.

WARNER, K. A., G. N. BOOKWALTER, J. J. RACKIS, M. E. HOCKRIDGE, D. H. HONIG, AND W. F. KWOLEK. Prevention of Rancidity in Experimental Rat Diets for Long-Term Feeding. Presented at the 65th Annual Meeting of American Association of Cereal Chemists, Chicago, IL, September 21-25, 1980.

WARNER, K. A., T. L. MOUNTS, J. J. RACKIS, AND W. J. WOLF. Relationships of Sensory Characteristics and Gas Chromatographic Profiles of Soybean Protein Products. Presented at the 65th Annual Meeting of American Association of Cereal Chemists, Chicago, IL, September 21-25, 1980.

4. Analytical and Structure Studies of Soybean Proteins (W. J. Wolf)

- a. Specific Objective: Continue development of a polyacrylamide gel electrophoretic "map" consisting of a plot of electrophoretic mobilities of soybean proteins as a function of gel concentration to provide information for optimal separation of the proteins and for characterizing them.

Progress: Preparative electrophoresis of soybean proteins was performed on agarose gels to achieve nonsieving, steady-state stacking conditions. After electrophoresis, the gels were sliced and the eluted proteins were subjected to Ferguson plot analysis. In this way, electrophoretic data were obtained for each of seven soy protein groups. The distinction between groups is based upon electrophoretic free mobility, Y_0 , while the proteins within a group differ in retardation coefficient, K_R . The Ferguson plot data were used to calculate gel concentrations which were optimum for electrophoretic resolution of the proteins in each Y_0 group.

- b. Specific Objective: Investigate sonication-effected soybean protein aggregation.

Progress: Water extract from defatted soybean flakes contains about 34% 7S proteins and less than 10% of aggregates greater than 15S. In a sonicated water extract, however, 7S decreased to 15% and aggregates increased to 35%. Sonication clearly promoted aggregation of the 7S proteins. Purified β -conglycinin also forms aggregates either by sonication or by heat treatment. However, the aggregates produced by heating appear to be different from those formed by sonication. Aggregates produced by sonication are stable at high ionic strength (0.5 μ). Formation of aggregates is favored at high protein concentration and low pH values (5-6). Once the proteins are sonicated, aggregation was found to be irreversible under conditions of dialysis in buffers of varied ionic strength or in distilled water at pH 5.0 or 7.0.

- c. Specific Objective: Evaluate thin-layer chromatography (TLC) of stachyose as a method for quantitating the amount of soy flour added to a wheat-soy composite flour.

Progress: TLC of soy flour extracts on kieselguhr-silica gel (1:4) coated plates with CHCl₃:MeOH:H₂O (1:1:1) gave good separations of stachyose, raffinose, and stachyose. Wheat flour extracts revealed a more complex oligosaccharide mixture than in soy flour; they contained raffinose and sucrose, several unidentified sugars, plus carbohydrate material that streaked from the origin through the position of stachyose. There was no clear evidence for stachyose and the slow-migrating sugars may be glucofructans. Interference of the presumed glucofructans was minimized by multiple ascent chromatography. Elution and colorimetric determination gave an apparent stachyose content of 0.06% for wheat flour whereas soy flour contains 70 times as much stachyose.

Quantitation of soy in wheat-soy composite flours by TLC appears feasible, but further refinement of the technique is needed. The absence of stachyose needs to be established and interference by glucofructans eliminated. This work was in cooperation with Western Regional Research Center (WRRC), SEA-AR, in support of USAID-WRRC PASA AG/TAB 231-11-76, "Improving the Nutritive Value of Wheat Foods."

Publications:

BRAUDE, G. L., A. M. NASH, W. J. WOLF, R. L. CARR, AND R. L. CHANEY. Cadmium and Lead Content of Soybean Products. *J. Food Sci.* 45 (1980):1187-1189, 1199.

ELDRIDGE, A. C., AND W. J. WOLF. Analysis of Soy Protein in Meat Products. In Proceedings World Soybean Research Conference II. Edited by Frederick T. Corbin, Westview Press, Boulder, CO, pp. 721-727. 1980.

NASH, A. M., AND W. J. WOLF. Aging of Soybean Globulins: Effect on Their Solubility in Buffer at pH 7.6. *Cereal Chem.* 57 (1980):233-236.

WANG, L. C. Soybean Protein Agglomeration: Promotion by Ultrasonic Treatment. Accepted for publication by the *J. Agric. Food Chem.* 1980.

WOLF, W. J. Soybean Proteins in Human Nutrition. In World Soybean Research Conference II: Proceedings, Edited by Frederick T. Corbin, pp. 767-780, Westview Press, Boulder, CO. 1980.

WOLF, W. J., AND F. L. BAKER. Scanning Electron Microscopy of Soybeans and Soybean Protein Products. *Scanning Electron Microscopy* 1980, III, 621-634.

Other Reports:

ANDERSON, R. L. Electrophoretic Analysis of Soy Protein Acid-Sensitive Fraction. Presented at American Association of Cereal Chemists' Meeting. Chicago, IL, September 1980.

ELDRIDGE, A. C. Determination of Soya Protein in Processed Foods. Presented at World Conference on Soya-Processing and Utilization, Acapulco, Mexico, November 9-14, 1980.

WANG, L. C. Soybean Protein Agglomeration: Promotion by Ultrasonic Treatment. Presented at Midwest Plant Physiologists Meeting, Marshall, IN, August 1980.

WOLF, W. J. Chemical and Physical Properties of Soybeans. Presented at Soyfoods Showcase, Soycrafters Association of North America, Urbana, IL, July 9-13, 1980.

WOLF, W. J. Progress and Future Needs for Research in Soya Protein Utilization and Nutrition. Presented at World Conference on Soya Processing and Utilization, Acapulco, Mexico, November 9-14, 1980.

5. Nutritional and Physiological Studies of Soybean Hemagglutinins (P.L. 480 Grant - Baroda University)

- a. Specific Objective: Isolate pure soybean hemagglutinins and study their nutritional and physiological effects on laboratory animals.

Final Report: Idli, a popular fermented food in India, is traditionally made from a mixture of rice and the legume, blackgram. Fermentation appears to involve four bacterial strains; namely, Leuconostoc mesenteroides, Lactobacillus fermenti, Lactobacillus delbrueckii, and Bacillus species. Soybeans can be substituted for blackgram without any significant effects on the organoleptic properties of the modified idli as compared to the traditional product. Rice-soy idli should have a better nutritional value than traditional idli because soybeans have higher protein and oil contents and contain more of the essential amino acids than blackgram. Soybean hemagglutinin, a glycoprotein that causes clumping of red blood cells in in vitro tests, is completely inactivated during the bacterial fermentation process and, therefore, poses no apparent problem as an antinutritional factor. Although feeding of unhydrolyzed soybean hemagglutinin to rats increased activities of proteases, hexaminidase, and α -D-mannosidase in the intestine, hydrolyzed agglutinin had no effect on these enzyme activities. Three bacterial enzymes responsible for hydrolysis of soybean hemagglutinin during the idli fermentation were isolated from a culture filtrate of Leuconostoc mesenteroides and characterized: protease, β -N-acetylglucosaminidase and α -D-mannosidase.

B. TECHNOLOGIES FOR INDUSTRIAL USES FOR
PLANT AND ANIMAL PRODUCTS

1. Chemical Modification of Soybean Oil and Its Derivatives (E. H. Pryde)

- a. Specific Objective: Prepare and characterize previously unknown hydrosilylated unsaturated fatty acid derivatives of soybean oil as part of a basic study for future technology based on renewable resources.

Progress: Hydrosilylation of methyl oleate with several silanes was studied. With methylbis(trimethylsiloxy)silane and chloroplatinic acid catalyst, a 57% yield (GLC) of product containing three major components was obtained. The largest of these components (41%) was isolated by fractional distillation. Nuclear magnetic resonance spectroscopy showed this product to be methyl methylbis(trimethylsiloxy)silyloctadecanoate. Mass spectroscopy confirmed this but did not locate the position(s) of the silyl moiety. Methylphenylsilane, used in fourfold excess, with chloroplatinic acid catalyst, gave 91% products (GLC), 83% of which was a single component. Extraction of the homogeneous catalyst or distillation in its presence caused reaction of the remaining silane hydrogen. Similarly, diphenylsilane apparently reacted through both silane hydrogens to give an intractable "dimeric" product. Catalyst concentration affected yield of products in hydrosilylation with dichloromethylsilane. Use of one-third the concentration of chloroplatinic acid reported as optimum increased yield from 67 to 84%. Distillation, crystallization, and column chromatography have effected only partial separation of product components from dichloromethyl- and triethoxysilane. Hydrosilylation of soybean oil with dichloromethylsilane resulted in addition of about one molecule of silane per double bond.

- b. Specific Objective: Prepare and characterize sulfur compounds of unsaturated fatty acids of soybean oil as part of a basic study on the mechanism of sulfurization of the extreme pressure lubricant additives formerly made from sperm oil.

Progress: Fatty acids and esters of soybean oil have been sulfurized and solubilized in a standard base mineral oil (Fisher 0-120) with surfactants. Five samples were submitted to the Southwest Research Institute for extreme pressure and wear scar tests. Extreme pressure results by the four-ball weld method (ASTM D-2596) showed values of 200, 250, 315, 500, and 600 kg. Previously sulfurized sperm whale in the same mineral base (Fisher 0-120) had been run at the Eastern Regional Research Center (ERRC) and had a weld point value of 220 kg. Wear scar index (ASTM D-2266) gave values of 0.91, 0.56, 0.74, 0.91, and 0.96 mm, respectively. The ERRC wear scar value for sulfurized sperm whale oil was 0.56 mm.

- c. Specific Objective: Synthesize and characterize model organosulfur compounds to give insight into the mechanism of the sulfurization reaction and into the mode of action in the performance of extreme pressure lubricant additives.

Progress: Octadecyl trisulfide has been synthesized and currently is being characterized by X-ray diffraction spectra in cooperation with the Naval Research Laboratory. Preliminary data indicate that the compound crystallized in the triclinic form and has a trans configuration. A mass spectrum showed a characteristic parent peak at 602 with an intense fragment at 570 indicating loss of sulfur. Other characteristic fragments were observed at 318, 286, 285, and 252. Raman spectra showed a strong S-S-S stretching vibration at 488 cm^{-1} for the crystalline state and a strong vibration at 492 cm^{-1} for the melt at 57°C . An infrared spectrum with a cesium iodide pellet had an absorption at 478 cm^{-1} . Nuclear magnetic resonance data showed a chemical shift downfield of 2.863 ppm for the α -methylene protons. This value is intermediate between the values of 2.65 and 2.91 found for the disulfide and tetrasulfide, respectively, and is consistent with literature values of other similar trisulfides.

- d. Specific Objective: Develop new information on microemulsions and micellar systems for hybrid fuels from both gasoline and diesel oil incorporating aqueous alcohol or other annually renewable fuels obtainable from agricultural resources as a means of conserving petroleum and energy.

Progress: Two-phase mixtures of diesel fuel and aqueous ethanol were microemulsified and data were collected on rheological properties, densities, water tolerance, and critical solution temperatures. For the detergentless system of diesel fuel, aqueous ethanol and 1-butanol, it was found that the relative viscosity varied directly with the volume percent of the dispersed water phase and closely followed Einstein's formula. With ionic systems, it was found that relative viscosities varied with increasing volume percent of dispersed water by values greater than those predicted by Brinkman and Roscoe for suspensions of diverse particle size. Water tolerances for ionic systems were greater than those observed with 1-butanol. The nature of the surfactant and its concentration determine to a large degree the microemulsion's physical properties.

Two diesel fuel aqueous ethanol formulas have been tested in engines by agricultural engineers at the University of Illinois. It was concluded that the engine developed full power with both fuels; the brake thermal efficiency was increased by 4 to 5% with both fuels; lower exhaust temperatures were observed; there was reduced smoke and carbon monoxide emission, but increased levels of unburned hydrocarbons were present in the exhaust; the detergentless

microemulsion was superior to the ionic microemulsion in ASTM properties; both fuels have flash points below the minimum specified for no. 2 diesel fuel; and the hybrid fuels do permit use of aqueous ethanol that may be of practical interest during periods of petroleum shortages.

- e. Specific Objective: Preparation and characterization of branched-chain fatty acids from sterculia oil as petroleum-sparing chemical intermediates.

Progress: Procedures were developed for making a product containing about 90% of branched-chain fatty acids from sterculia oil. The procedures included rearrangement of the cyclopropenoid ring to conjugated dienes containing methylene- and methyl-branches with rhodium catalyst, hydrogenation of the conjugated dienes with the same catalyst, and recrystallization of the crude branched-chain fatty acids with mixed solvent of ethanol and water (80/20, v/v). In order to investigate the characteristic properties of these acids, the esters of the branched-chain fatty acids with 2-ethylhexyl alcohol and trimethylolpropane were prepared, and lubricant wear (mini four-ball test), AOM, and other tests are in progress.

Publications:

SCHWAB, A. W., L. E. GAST, AND H. E. KENNEY. Tetrasulfide Extreme Pressure Lubricant Additives. U.S. Patent 4,218,332. August 19, 1980.

SCHWAB, A. W., R. D. GILARDI, AND J. L. FLIPPEN-ANDERSON. Configuration of Octadecyl Tetrasulfide. Phosphorous and Sulfur. In press.

Other Reports:

BORUFF, P. A., A. W. SCHWAB, C. E. GOERING, AND E. H. PRYDE. Engine Evaluation of Diesel Fuel-Aqueous Ethanol Microemulsions. Presented at the 1980 Winter Meeting, American Society of Agricultural Engineers, Chicago, IL. December 4, 1980. Paper No. 80-1523.

PRYDE, E. H. Vegetable Oils as Diesel Fuel: An Overview. Presented at the Seminar, Vegetable Oil for Diesel Fuel, Peoria, IL. September 25, 1980.

PRYDE, E. H. Vegetable Oils for Fuels. Presented at the 21st Annual Corn Dry Milling Conference, Peoria, IL. June 3-4, 1980.

PRYDE, E. H. Vegetable Oils for Fuels. Presented at the NCR-114 Committee (Crop Residues as an Alternate Energy Source) Meeting, Peoria, IL. June 30-July 1, 1980.

PRYDE, E. H. World Fats and Oil Situation. Presented at the symposium "New Sources of Fats and Oils," American Oil Chemists' Society Meeting and ISF/AOCS Congress, New York, NY. April 27-May 1, 1980.

C. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. High-Temperature Soybean Cooking Oils for the Export Market (E. N. Frankel)

- a. Specific Objective: Study the kinetics and mechanism of continuous high pressure hydrogenation of soybean oil with copper-based and other catalysts to provide a technological basis for expanding the export market for edible soybean oil, margarine, and shortening products.

Progress: An infrared (IR) spectrometer of the "attenuated total reflectance" (ATR) type, a refractive index (RI) instrument, and an automatic gas chromatograph (GC) were connected to a laboratory continuous hydrogenation apparatus to monitor the reaction. An oil-catalyst (copper chromite) mixture was pumped through stainless steel tubing (ca. 40 ft. X 1/8 in.) while mixing concurrently with hydrogen. Pressure was controlled at ca. 210 psi and temperature was varied from ca. 170°C to 210°C. Under present operating conditions, GC analysis requires 10 to 14 minutes, RI approximately 6 minutes, and IR gives almost instant response. Thus, IR analyses can be used for immediate adjustments of hydrogenation followed by confirmation with the RI and GC measurements.

Ancillary apparatus (consisting of transducers, recorders, and alarms) for high-pressure continuous hydrogenation have been installed for testing under operating conditions.

- b. Specific Objective: Develop knowledge concerning the use of Ziegler-type catalysts in batch and continuous hydrogenation of soybean oil. The goal is to improve nutritional quality of consumer products.

Progress: The soluble complex catalyst formed by reacting copper stearate with triethyl aluminum was employed for the selective hydrogenation of soybean oil. This homogeneous catalyst is more active than copper-chromite. The activity was enhanced by the addition of silica, alumina or titania to the reaction mixture. Ethyl alcohol in small amounts also improved the activity, but hydrogenation was retarded when increased amounts were added. More active homogeneous catalysts resulted when triethyl aluminum was replaced by tri-isobutyl aluminum, tri-n-hexyl aluminum, tri-n-octyl aluminum, or tri-n-decyl aluminum. Among other organometallics tested, diethyl magnesium formed an active catalyst. The linolenate

selectivity of these homogeneous catalysts was much less (3.5) than normally achieved with copper-chromite catalyst (10). Isomerization with the homogeneous catalysts as measured by the percentage of trans-isomers formed was similar to that of heterogeneous catalysts ($\% \text{ trans}/\Delta \text{ I.V.} = 0.6-0.7$).

Addition of triethyl silane to copper stearate resulted in an active heterogeneous catalyst for the hydrogenation of soybean oil. The linolenate selectivity of this catalyst (2.4 to 3.9) was much lower than that obtained with copper chromite. Unlike copper-chromite catalyst, triethyl silane-activated copper formed stearate during hydrogenation. Both silica and alumina increased catalyst activity. Linoleate selectivity improved slightly in the presence of alumina.

- c. Specific Objective: Study effect of higher than normal catalyst concentrations and catalyst reuse on hydrogenation rates and products.

Progress: Operating conditions were regulated to hydrogenate soybean oil to an iodine value of 114. Catalyst was removed from the oil using a centrifuge, and small amounts of fresh catalyst were replaced to carry out subsequent reactions. The products were evaluated for activity and linolenate selectivity of the catalyst and for trans formation. By recycling the catalyst 10 times with 1% copper, the activity was retained if 20% of the used catalyst was replaced with fresh catalyst each time. Increasing the catalyst concentration to 2%, only 10% make-up catalyst was needed to retain the activity. In continuous hydrogenations using fresh catalyst each cycle, 1-2 g of catalyst are needed per 100 g of oil. However, in a recycling continuous operation, the catalyst needed is only about 0.2 g per 100 g of oil. There is, therefore, about tenfold saving in catalyst consumption by continuous hydrogenation with a 20% make-up with fresh catalyst.

- d. Specific Objective: Evaluate room odor characteristics of processed soybean oils to improve their acceptability and interchangeability in domestic and world markets.

Progress: Unhydrogenated and hydrogenated soybean oils were prepared with additives, singly, and in combination. Additives used were citric acid, BHA, BHT, TBHQ, methyl silicone, and anoxomer (a polymeric antioxidant).

- e. Specific Objective: Isolate and identify materials contributing to odor of soybean oil on storage and use in deep-fat cooking and salad oils.

Progress: Volatiles from oxidized soybean oil were collected at dry ice temperature both in hexane solution and alone. These

volatiles were fractionated by high-performance liquid chromatography with hexane or aqueous alcohol solvents. However, the odors in the collected fractions were too weak for reliable organoleptic characterization over the odor of the solvents. Also, rechromatography of collected fractions showed that the volatile compounds were unstable. Capillary gas chromatography of head gas from oxidized oils also separated many components but results were only poorly reproducible. Work toward this objective was discontinued.

- f. Specific Objective: Evaluate the effect of packaging on the storage stability of soybean oils.

Progress: Soybean and hydrogenated soybean oils are packaged commercially in a variety of glass and plastic bottles with and without nitrogen in the head space. Optimum packaging specifications include amber glass bottles and nitrogen blanketing. The effect of plastic packaging and air in the headspace on soybean oil quality was investigated by exposing oils to accelerated light exposure, temperature, and long-term ambient storage (up to 6 months at 77°F). In all cases, no differences in flavor scores were noted between oils packaged in clear glass and polyvinyl chloride (PVC) bottles. Therefore, the packaging of soybean and hydrogenated soybean oils in clear plastic bottles is not detrimental to the quality of the product. However, amber bottles still afforded the best protection against light exposure.

Publications:

BITNER, E. D., T. L. MOUNTS, AND H. J. DUTTON. Monitoring of Oil Composition by an Automated System for Esterification--GC Analysis. *J. Am. Oil Chem. Soc.* 57 (1980):209-212.

KORITALA, S. Selective Hydrogenation with Copper Catalysts: V. Kinetics and Mechanism at High Pressure. *J. Am. Oil Chem. Soc.* 57 (1980):293-298.

KORITALA, S. Method of Enhancing Activity of Homogeneous Ziegler-type Copper Catalysts. U.S. Patent Application filed 8-8-80.

KORITALA, S. AND E. N. FRANKEL. Selective Conjugation of Soybean Esters to Increase Hydrogenation Selectivity. *J. Am. Oil Chem. Soc.* In press.

KORITALA, S., J. P. FRIEDRICH, AND T. L. MOUNTS. Selective Hydrogenation of Soybean Oil. X. Ultra High Pressure and Low Pressure. *J. Am. Oil Chem. Soc.* 57 (1980):1-5.

MOUNTS, T. L. Hydrogenation Practices. "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 131-144, 1980.

SCHOLFIELD, C. R. Argentation High-Performance Liquid Chromatography of Methyl Esters. J. Am. Oil Chem. Soc. 57 (1980):331-334.

Other Reports:

KORITALA, S. AND E. N. FRANKEL. Selective Conjugation of Soybean Esters to Increase Hydrogenation Selectivity. Presented at the symposium of the North Central Section of the American Oil Chemists' Society, Chicago, IL. March 26, 1980.

MOUNTS, T. L., K. WARNER, AND G. R. LIST. Flavor and Oxidative Stability of Hydrogenated and Unhydrogenated Soybean Oil: Effect of Tertiary Butyl Hydroquinone. Presented at ISF-AOCS Meeting, New York, April 27-May 1, 1980.

MOUNTS, T. L. Future Trends in the Selective Hydrogenation of Vegetable Oils. Presented at Oils and Fats Group Seminar, The Chemical Society of London, London, England, September 15, 1980 and Symposium on Hydrogenation of Oils, Rimini, Italy, September 29, 1980.

SCHOLFIELD, C. R. Composition of Soybean Lecithin. Presented at ISF-AOCS Meeting, New York, April 27-May 1, 1980.

SNYDER, J. M., T. L. MOUNTS, C. R. SCHOLFIELD, AND H. J. DUTTON. Laboratory-scale Continuous Hydrogenation. Effect of Pressure. Presented at ISF-AOCS meeting, New York, April 27-May 1, 1980.

WARNER, K. Correlation of Sensory Data and Gas Chromatographic Volatiles. Profiles of Soybean Oil and Protein. Presented at Soybean Research Review, Peoria, IL, May 15-16, 1980.

2. Soybean Oil Quality for Export Markets (E. N. Frankel)

- a. Specific Objective: Conclude study of effects on oil quality of storing soybeans damaged by harvesting equipment.

Progress: Final samples were taken at 22 months and analyzed for critical oil quality. Statistical significance of effects of storage parameters was determined.

The free fatty acid (FFA) content was directly related to the refining losses during processing of the oil. In the present study, FFA values ranged from 0.26 to 0.53%, which is nearly normal in commercially extracted crude oils (0.3 to 0.7%). Iron is known to be an active catalyst for oxidative deterioration. Crude soybean oil iron content ranged from 0.55 to 1.30 ppm. This amount is near the normal range of 1 to 3 ppm. There was no statistical correlation of FFA and iron contents with storage time, variety, or harvest damage level.

Analyses of crude oil for phosphorus provide valuable information about phospholipids which are progressively broken down as soybeans are damaged. Such phospholipids are difficult to remove and they increase refining losses. The phosphorus content of the oil from soybeans in this study was somewhat higher initially and after 4 months storage than after 23.5 months storage. Analyses of oil from soybeans that had been stored for periods of 7.5 and 10.5 months indicated that the extractable phosphorus had declined; however, the phosphorus content was within the 500 to 700 ppm range normally found in soybean oil. These variations may be attributable to the life processes continuing during storage of the seed. After 23.5 months in storage, phosphorus analyses of the extracted crude oil indicated severe deterioration of the phospholipids. This deterioration appears to be related only to the length of time that the soybeans are stored. No correlation was found between phosphorus content and variety or harvest damage level.

- b. Specific Objective: Develop further knowledge concerning the deterioration of oil quality during export shipment of soybeans.

Progress: Previous studies have provided evidence that shipments of soybeans from the United States to Europe result in the deterioration of crude oils extracted from these exported beans. To further investigate this problem, a cooperative program was implemented with Professor Lowell Hill, University of Illinois, Urbana, Illinois. Professor Hill provided identity-preserved samples from two shipments leaving the United States at Beaumont, Texas and arriving at Tilbury, England. The origin samples of each shipment were combined and analyzed as a single composite fraction. The destination samples were taken at different locations within the ships hold, since the holds of cargo ships are very large and temperature and moisture gradients surely must exist. The destination samples were then analyzed as individual samples and compared to the origin samples.

Our analyses confirmed that export shipments caused deleterious effects on crude oil quality. Crude oils extracted from destination samples showed an increase in free fatty acid content compared to that of origin samples. Destination samples showed a decrease in phosphorus content of the crude oils. This reduction in phosphorus would indicate formation of non-hydratable phosphatides during export. Oxidative deterioration occurred also as shown by increases in peroxide values. Split content showed an increase from origin to destination.

- c. Specific Objective: Complete design and construction of laboratory adiabatic storage silo. Study the effects of parameters of soybean storage on critical oil quality factors.

Progress: An adiabatic reactor and its controller were improved. In preliminary experiments, approximately 200-g samples of soybeans tempered to ca. 19% moisture were placed in reactor. The first trial beans did not intrinsically heat and the experiment was terminated after 10 days. In a second trial, a "fine" adjustment of the temperature controller was made after 10 days since the room temperature was apparently affecting the heating. The beans began intrinsically heating and continued until the temperature reached 45°C. Beans appeared dark in color and had a "dirt" odor. Moisture was ca. 18%. Extraction and analyses of the oils are now underway.

- d. Specific Objective: Determine the composition and structure of the deleterious nonhydratable soybean phosphatides which are formed during soybean and oil storage and transportation.

Progress: Various procedures were evaluated to isolate nonhydratable soybean phosphatides (NHP) from crude oils. The hydratable phospholipids were isolated from the degummed oil by extraction with aqueous methanol and petroleum ether followed by liquid chromatography on silica gel. The resulting nonhydratable phosphatides were analyzed by thin-layer and high-pressure liquid chromatography and identified as follows: phosphatidic acid, lysophosphatides, phosphatidylethanolamine, and phosphatidylserine. Pigments were also present in the NHP fraction.

- e. Specific Objective: Complete study of residual metal content of commercial edible oil products.

Progress: Based upon processors concern regarding transition metals, methods of direct sampling were developed for nickel, copper chromium, and iron. Analyses were carried out by flameless atomic absorption spectrometry using method of additions with organometallic compounds in salad oils. Of five partially-hydrogenated cooking oils and the fats recovered from five margarines, nickel occurred at a level below the limit of detection (0.16 ppm Ni) in all but two cooking oils which contained 0.16 and 0.25 ppm Ni. Analyses of five commercial shortenings gave values ranging from 0.6 to 2.8 ppm Ni. The unexpectedly low values in liquid shortenings have been confirmed by alternate methods involving ashing. Good agreement in nickel analysis was also obtained by independent analyses in cooperation with CSF and an industrial laboratory. Copper, chromium, and iron, absent from liquid shortenings, were found in specific margarine oils. Levels were 0.054-0.075 ppm Cu, 0.012-0.014 ppm Cr, and 0.56-0.72 ppm Fe. Applicable limits of detection were 0.053 ppm Cu, 0.007 ppm Cr, and 0.49 ppm Fe. It is concluded that present technology effectively removes residual nickel catalyst from partially hydrogenated soybean oils used for salad-cooking oils and margarines, but not from more highly hydrogenated fats used in plastic shortenings.

- f. Specific Objective: Study conditions that promote the formation of nonhydratable phospholipids during storage, transportation, and handling of exported soybeans.

Progress: Preliminary experiments aimed at defining conditions that promote the formation of nonhydratable phosphatides (NHP) during storage were completed. Soy beans were tempered to 14.6% moisture and stored at 81°F for up to 2 months. After cracking, flaking, and extraction, the crude oils were degummed with water. Over 50% of the phosphorus could not be removed by hydration. Therefore, the formation of NHP is fairly rapid. Beans stored for 1 month produced crude oil having a residual phosphorus content of 210 ppm compared to 485 ppm in the unstored crude. Such oil would be discounted in trading channels. The formation of NHP in soybeans results apparently from storage at high moisture levels and elevated temperatures.

- g. Specific Objective: Complete writing, editing, and subject indexing for the "Handbook of Soy Oil Processing and Utilization" being developed in cooperation with the American Soybean Association (ASA) and submit to publisher.

Progress: The Handbook was published in late October in time for distribution by ASA to the registrants at the World Conference on Soya Processing and Utilization at Acapulco, Mexico, November 9-14, 1980. The ASA will also distribute the Handbook to various Food Science Departments at universities throughout the world. The Handbook will also be translated into Spanish.

- h. Specific Objective: Acquire knowledge concerning the effect on oil quality and composition of extracting of soybeans with supercritical fluids.

Progress: Full-fat soy flakes were readily extracted with supercritical CO₂ at a pressure of 5000 psi and 50°C to a residual oil content of less than 0.5%. The equilibrium solubility of the oil in CO₂ under these conditions was 1.2-1.4% by weight. Because supercritical CO₂ has the density of a liquid and the diffusivity of a gas, equilibrium can be established rapidly to afford high flow rates. The oil was separated from the gas phase by reducing the pressure. The solvent-free oil was compared to hexane-extracted oil from the same beans. Free fatty acid, trace metals, peroxide value, fatty acid composition, chromatographic refining loss, and unsaponifiable content were similar for the two oils. However, phosphorus content of the CO₂-extracted oils was significantly lower and was comparable to a degummed crude. Decreased solubility of phospholipids is therefore indicated. A new project is being prepared to increase effort on this promising new technology.

Publications:

BRAUDE, G. L., A. M. NASH, W. J. WOLF, R. L. CARR, AND R. L. CHANEY. Cadmium and Lead Content of Soybean Products. *J. Food Sci.* 45 (1980):1187-1189, 1199.

BREKKE, O. L., T. L. MOUNTS, AND E. H. PRYDE. Summary and Recommendations. In: "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 551-560 (1980).

ERICKSON, D. R., E. H. PRYDE, O. L. BREKKE, T. L. MOUNTS, AND R. A. FALB, Editors, "Handbook of Soy Oil Processing and Utilization," published jointly by the American Soybean Association and the American Oil Chemists' Society, Champaign, IL (1980).

HAMERSTRAND, G. E. AND G. R. LIST. Addendum to Partially Hydrogenated Winterized Soybean Oil: Cost Estimates. In: "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 214-216 (1980).

LIST, G. R. Special Processing for Off Specification Oil. In: "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 355-376 (1980).

LIST, G. R., J. M. AVELLENADA, AND T. L. MOUNTS. Degumming of Soybean Oil: Effect of Operational Conditions on Lecithin Removal and Quality. *J. Am. Oil Chem. Soc.* In press.

LIST, G. R. AND D. R. ERICKSON. Storage, Handling, and Stabilization. In: "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 267-353 (1980).

LIST, G. R. AND T. L. MOUNTS. Partially Hydrogenated-Winterized Soybean Oil. In: "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 193-214 (1980).

MOUNTS, T. L. Chemical and Physical Effects of Processing Fats and Oils. *J. Am. Oil Chem. Soc.* In press.

MOUNTS, T. L. Recent Developments in Vegetable Oil Processing Technology. *Grasa Y Aceites* 31 (1980):37-49.

MOUNTS, T. L. AND K. WARNER. Evaluation of Finished Oil Quality. In: "Handbook of Soy Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 245-266 (1980).

MUSTAKAS, G. C. Recovery of Oil from Soybeans. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 49-65 (1980).

PRYDE, E. H. Soybeans vs. Other Vegetable Oils as Sources of Edible Oil Products. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 1-11 (1980).

PRYDE, E. H. Composition of Soybean Oil. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 13-31 (1980).

PRYDE, E. H. Physical Properties of Soybean Oil. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 33-47 (1980).

PRYDE, E. H. Sources of Information. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 561-577.

PRYDE, E. H. Nonfood Uses for Soybean Oil. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 459-481.

PRYDE, E. H. AND O. L. BREKKE. Environmental Concerns: An Annotated Bibliography. In: "Handbook of Soybean Oil Processing and Utilization," Edited by D. R. Erickson, E. H. Pryde, O. L. Brekke, T. L. Mounts, and R. A. Falb, pp. 527-550 (1980).

Other Reports:

LIST, G. R. Partially Hydrogenated-Winterized Soybean Oil. Presented at AOCS-ISF Meeting, New York, NY, April 27-May 1, 1980.

LIST, G. R., J. M. AVELLENADA, AND T. L. MOUNTS. Degumming of Soybean Oil: Effect of Operational Parameters on Lecithin Removal and Quality. Presented at AOCS-ISF Meeting, New York, NY, April 27-May 1, 1980.

MOUNTS, T. L. Effects of Storage, Handling, and Export Shipment of Soybeans on Crude Oil Quality. Presented at General Collaborators Conference, Peoria, Illinois, October 20-21, 1980.

PAULSEN, M. R., NAVÉ, W. R., MOUNTS, T. L., AND GRAY, L. E. Storability of Harvest-Damaged Soybeans. Presented at Am. Soc. of Agric. Engineers, San Antonio, TX. June 15-18, 1980.

3. Heavy Metals in Soybeans Grown on Sewage Sludge-Amended Soil (Cooperative Agreement - Western Illinois University)

- a. Specific Objective: Investigate the effect on soybean yield and the modification of trace metal content of soybeans, meal, and oil from the use of municipal sewage sludge for fertilization.

Progress: Experimental plots of soybeans were grown on sludge-amended soil at Western Illinois University. Municipal sewage sludge was obtained and used as a soil nutrient in three replications of plots, 25 X 100 ft each of the following conditions for evaluation: (a) no added nutrients but with an amount of water equal to that added with sludge treatment of other plots; (b) two plots with 50 tons/acre (dry basis) of municipal sewage sludge; and (c) two plots with 100 tons/acre (dry basis) of municipal sewage sludge. Seeds were planted and the crop was harvested for processing and analysis.

D. HUMAN REQUIREMENTS FOR NUTRIENTS

1. Biochemical Behavior of Isomeric Fats in Hydrogenated Soybean Oil (E. A. Emken)

- a. Specific Objective: Studies on the metabolic fate of trans-13- and cis-13-octadecenoic acid in human blood lipids. This work contributes basic information with which to evaluate the nutritional value of hydrogenated soybean oil.

Progress: Mixture of triglycerides containing deuterated trans-13-, cis-13-, and cis-9-octadecenoic acids were fed to two young adult male subjects and blood samples were drawn at 9 intervals over a 48-hour period after feeding. Each sample was separated into plasma, red cell, chylomicron and very low density, low density, and high density lipoprotein fractions. Each fraction was further separated into 8 to 10 individual lipid samples, derivatized, and analyzed by mass spectroscopy. Selectivity values were calculated for each sample to compare the relative incorporation of the cis- and trans-13-octadecenoic acid isomers to cis-9-octadecenoic acid. Results indicate the 13-octadecenoic acid isomers in hydrogenated soybean oil are over 98% absorbed. Very low amounts of these isomers were incorporated into plasma and cholesteryl ester fractions. Incorporation of both the cis- and trans-13-octadecenoic acid isomers into plasma and lipoprotein phospholipids samples was greater than for cholesteryl ester samples but total incorporation of the 18 position isomers was significantly less in all samples than for cis-9-octadecenoic acid. Distribution of the 13-octadecenoic acid isomers was significantly different for the 1 and 2 acyl position of phosphatidylcholine. Individual lipid selectivity values were discovered to also be dependent on both the lipoprotein class and individual lipid class. This work was in cooperation with St. Francis Medical Center, Peoria, Illinois.

- b. Specific Objective: Synthesize triglycerides of dideuterated geometric isomers of 12,15-octadecadienoic acid. These compounds are to be used in metabolism studies on the nutritional value of hydrogenated soybean oil.

Progress: Multigram amounts of the four geometrical (i.e., cc, ct, tc, and tt) methyl 12,15-octadecadienoate-9,10-d₂ isomers were prepared by stereoselective control of the Wittig reaction. The mixtures obtained (cc with tc and ct with tt) were separated by partial silver resin chromatography. The esters were converted to triglycerides for use in human feeding studies. Melting points of the acids, esters, and triglycerides were determined by differential thermal analysis. ¹H and ¹³C NMR spectroscopy of these compounds confirmed the assigned configurations.

- c. Specific Objective: Expand silver resin chromatographic capabilities to allow separation of fatty acid mixtures and of triglyceride mixtures.

Progress: Replacement of the sulfonic acid protons of XN1010 resin with sodium ions before silver ion incorporation prevented transesterification and interesterification when fatty acids were separated by methanol elution. Acetone was found to be the most universally applicable solvent for the separation of mixtures of fatty acids, mixtures of fatty esters and mixtures of triglycerides. The use of acetone is mandatory for the latter class of compounds since they are only slightly soluble in methanol. Neutralization of the resin with sodium ions prevented acid catalysed condensation of the acetone at low resin silver ion levels.

- d. Specific Objective: Evaluate synthetic schemes for preparation of deuterated octadecadienoic acid, palmitate-d₂, and stearate-d₆.

Progress: An eight-step synthesis was developed which gave an overall yield of ~30% methyl cis-9,cis-12-octadecadienoate-16,16,17,17-d₄. The preparation utilized easily obtainable starting materials. Tris(triphenylphosphine)chlororhodium (I) catalyst was used for the deuterium isotope incorporation. The double bond in the 9 position was generated by the Wittig coupling of 1-non-3-enyl-d₄ triphenylphosphonium bromide to methyl 8-formyloctanoate. The 1-non-3-enyl-d₄-bromide was prepared by the acetylenic coupling of iodopentane-d₄ and 3-butyn-1-ol. Other methods for synthesis of the intermediates and final product were evaluated. Partial argentation resin chromatography was used to remove about 9% trans,cis-, cis,trans-, and trans,trans-isomers also produced. The final product was greater than 98% pure by capillary gas chromatography (OV-101, 24 meter). Mass spectrometry indicated 96%-d₄.

Synthesis of palmitate-d₂ and stearate-d₆ from petroleum-based intermediates was determined to be a more facile route than synthesis from natural fatty acid precursors.

- e. Specific Objective: Prepare methyl trans-10-octadecenoate-10(11)-³H.

Progress: Methyl trans-10-octadecenoate-10(11)-³H was prepared from triglycerides containing a mixture of cis-and trans-10-octadecenoic acids. Bromination followed by debromination yielded crude octadec-10-ynoic acid. The methyl ester was prepared with BF₃-MeOH. Methyl octadec-10-ynoate was reduced with tritiated water and Lindlar catalyst to methyl-10-octadecenoate-10(11)-³H. Isolation of pure cis-10-isomer was accomplished by silver-resin chromatography. Isomerization to the trans-10-isomer with sulfinic acid catalyst followed by silver-resin chromatography gave pure methyl trans-10-octadecenoate-10(11)-³H.

- f. Specific Objective: To identify volatiles from heated vegetable or simulated cooking oils and correlate the volatile with particular (fishy, painty) heated oil room odors.

Progress: The triglycerides of individual fatty acids, in soybean oil have been subjected to cooking oil conditions and ensuing volatiles were collected and identified. Results of volatiles identified in heated tristearin, triolein, trilinolein, and hydroperoxides of methyl oleate have been published. The procedures used in the above studies have been extended to tri(cis-9,cis-15-octadecadienyl)glycerol, a component of hydrogenated soybean oil. Unusual aldehydes of or similar to those derived from the isolinolein are being regenerated from their DNPH's and are being used to confirm the identity of the various volatile aldehydes.

- g. Specific Objective: Develop new techniques in the determination of deuterium-labeled compounds and analyze lipids extracted from human blood.

Progress: A new chemical ionization technique using a mixture of methane and isobutane as reagent gases has been developed and applied to blood lipids from a human subject. The new chemical ionization technique has produced a 20-fold improvement in sensitivity over the older electron impact ionization method.

- h. Specific Objective: Explore the distribution of dietary positional octadecenoic acid isomers in total lipids from human tissue.

Progress: This study was designed to obtain information on the extent of incorporation and possible accumulation of fatty acid positional isomers in humans which occurs during long-term dietary

consumption of hydrogenated fats. Techniques for the isolation of fatty acid geometrical isomers from human tissue were developed and evaluated. Improvements in existing technology were necessary due to the low levels of trans and positional isomers in human tissues. A method of quantitation and validation of cis-trans isomer separation and recovery was developed using radiolabeled elaidate and oleate tracers as internal standards. Modifications in the methodology of double bond position analysis were made in order to accomodate the small sample size available in these studies. These modifications were evaluated through analysis of known mixtures of positional isomers. Preliminary analyses have been carried out on human tissues obtained during autopsies performed on six subjects. The double bond position and configuration of the octadecenoate fraction of total lipids extracted from heart, brain, liver, aorta, and adipose tissue was determined. The level of trans octadecenoate in the tissues was determined by both direct GLC and by GLC after silver nitrate TLC. Results obtained by both methods indicated an average of 2.65% trans 18:1 in the total tissue lipids. The human tissues were found to contain trans-isomers having double bonds between the 6 and 15 positions while cis double bonds were found to occur between the 6 and 14 positions. These results demonstrate that human metabolism does not discriminate absolutely against incorporation of these unusual dietary positional isomers into tissue lipids.

- i. Specific Objective: Identify molecular structures using gas chromatography-mass spectrometric analysis in collaboration with scientists from within and outside NRRC.

Progress: A wide range of compounds have been analyzed and include: deuterated intermediate products, reductones, indenones, and isomaltol derivatives. Several surveys of mycotoxin-contaminated corn were run to determine the presence of vomitoxin, T-2 toxin and four other mycotoxins.

- j. Specific Objective: Separation of cyclic terpene resin esters.

Progress: Assisted Dr. Duane F. Zinkel, USDA Forest Products Laboratory, Madison, Wisconsin, in the separation of poly cyclic resin esters. From a mixture of seven polycyclic isomers, four were separated by acetone elution on a 72 X 0.6 cm column packed with silver saturated/sodium ion 80/100 mesh Amberlyst XN 1010 resin.

- k. Specific Objective: Complete competitive deposition studies involving trans-12- and cis-9-octadecenoic acids in the laying hen.

Progress: Specific activities were determined on each egg neutral lipid and phospholipid component and on the fatty acids in each acyl position of these lipids in order to complete the study of

competitive incorporation of trans-12- and cis-9-octadecenoic acids to laying hens.

1. Specific Objective: Compare the distribution of trans-8-octadecenoic acid to cis-9-octadecenoic acid in egg yolk lipids.

Progress: Mixtures of methyl trans-8-octadecenoate-8(9)-³H and methyl cis-9-octadecenoate-10-¹⁴C were fed to three laying hens and analyses of yolk lipid components were initiated. Preliminary data show that only phosphatidyl ethanolamine (PE) incorporated more trans-8- than cis-9-octadecenoate. Acyl positional analyses indicate that the trans-8-isomer was preferentially esterified at the primary positions in triglycerides (TG), phosphatidyl choline (PC), and PE but was almost totally excluded from the 2-position of PE and PC. Cholesteryl esters (CE) showed marked preference for the cis-9-octadecenoate.

- m. Specific Objective: To cooperate with Center and other scientists in research related to lipid oxidation or research requiring computerized mass spectrometry-gas chromatography.

Progress: Cooperative studies with E. Frankel and W. Neff of Oilseed Laboratory on comparing volatiles from autoxidized and photosensitized methyl esters and with J. W. Newton of Fermentation Laboratory on nitrogen fixation by an algal symbiont were completed. A request from the Department of the Treasury for aid in identification of volatiles released during the curing of freshly printed currency was received. A report of our contributions toward resolving the Treasury Department's problem is near completion.

- n. Specific Objective: Modernize mass spectrometer and computer systems to increase efficiency, reduce down time, and extend instrument capabilities.

Progress: The PDP-8 computer has been replaced with a modern INCOS 2000 data system. A Bendix GC and a glass jet separator have replaced the aging Packard GC and the membrane separator which limited the temperature of compounds that could be analyzed.

- o. Specific Objective: Evaluate methods for the synthesis of (E,Z)-3,5-tetradecadienoic acid (megatomic acid). Megatomic acid is the pheromone of the black carpet beetle [Attagenus megatoma (F)], a destructive pest of stored grain. The other three related geometrical isomers are not active, but the (Z,Z)-isomer is a pheromone for a related beetle.

Progress: A stereospecific synthesis of megatomic acid was devised utilizing the capabilities of the Wittig reaction. Alkylation of 2-propyn-1-ol with 1-bromoocctane gave 2-undecyn-1-ol which could be

oxidized to 2-undecynal with either pyridium chlorochromate or chromic acid. Reaction of either 2-carboxyethyl- or 2-carbomethoxyethyl-triphenylphosphonium bromide by Wittig reaction gave complex mixtures of products. Isolation of 4-hydroxy-2-tetradecen-5-ynoic acid indicated a different reaction course than envisioned. Wittig reaction between 3-(2-tetrahydropyranloxy)propyltriphenylphosphonium bromide and 2-undecynal has surmounted this difficulty but introduced additional steps in the synthesis.

- p. Specific Objective: Synthesize the triglyceride of (Z)10-octadecenoic-13,14-d₂ acid for use in human metabolism experiments.

Progress: (Z)10-Octadecenoic-13,14-d₂ was prepared by Wittig reaction of 1-octyl-3,4-d₂-triphenylphosphonium iodide and methyl 10-oxodecanoate. The triglyceride was prepared from the corresponding octadecenoyl chloride in quantities sufficient for feeding studies. Capillary gas chromatography showed absence of (E)-isomer. The isotopic purity was 89%.

- q. Specific Objective: Initiated work on comparing the distribution of cis and trans-11-octadecenoic acid to oleic acid in human blood lipids.

Progress: A young adult male subject was fed a mixture of triglycerides containing deuterium labeled trans-11-, cis-11-, and cis-9-octadecenoic acid. Blood samples were drawn at 9 intervals over a 48-hour period. The specific lipid classes in plasma, red cell, platelet, chylomicron, and very low, low, and high density lipoproteins have been separated and derivitized for analysis of deuterium labeled fats by mass spectroscopy. This work was in cooperation with Dr. Gulley, St. Francis Medical Center, Peoria, Illinois.

- r. Specific Objective: Cooperate with Dr. Parsons, Hospital for Sick Children, University of Toronto, Canada, on fat absorption studies in humans.

Progress: Three normal adult male subjects were administered solutions containing mixtures of deuterated stearic, oleic, elaidic, and linoleic acid. Samples from the intestinal tract and blood lipids were obtained. Individual lipid fractions were isolated and derivatized. Analysis by mass spectroscopy is in progress to determine the relative absorption of these fats. These data will establish baseline values for future fatty acid absorption studies with cystic fibrosis patients.

Publications:

ADLOF, R. O., AND E. A. EMKEN. A Versatile Procedure for the Preparation of Palmitic Acid-d₂ and Stearic Acid d-6. J. Labelled Compd. Radiopharm. In press.

ADLOF, R. O., H. RAKOFF and E. A. EMKEN. Partial Argentation Resin Chromatography (PARC) I: Effect of Percent Silver on the Elution and Separation of Methyl Octadecadienoate Isomers. J. Am. Oil Chem. Soc. 57 (1980):273-275.

ADLOF, R. O. AND E. A. EMKEN. Partial Argentation Resin Chromatography (PARC) II: Separation of Saturated and Mono-, Di-, Tri-, and Tetraenoic Fatty Esters. J. Am. Oil Chem. Soc. 57 (1980):276-278.

ADLOF, R. O. AND E. A. EMKEN. Partial Argentation Resin Chromatography (PARC) III: The Effects of Sodium Ion Incorporation and Solvent on the Separation of Mixtures of Fatty Acids, of Fatty Esters, and of Triglycerides. J. Am. Oil Chem. Soc. Accepted 1980.

DeJARLAIS, W.J. AND E. A. EMKEN. A Convenient Synthesis of ω -Acetylenic Acids. Synth. Commun. 10 (1980):653-660.

DUTTON, H. J. Incorporation of Isomeric Fatty Acids in Egg and Human Blood Lipids. J. Am. Oil Chem. Soc. 57 (1980):122A, Abstract 62.

EMKEN, E. A., H. J. DUTTON, W. K. ROHWEDDER, HENRY RAKOFF, R. O. ADLOF, R. M. GULLEY, AND J. J. CANARY. Distribution of Deuterium-Labeled *cis* and *trans*-12-Octadecenoic Acids in Human Plasma and Lipoprotein Lipids. Lipids 15 (1980):864-871.

EMKEN, E. A. Nutritive Value of Soybean Oil. In: Handbook of Soy Oil Processing and Utilization, edited by David R. Erickson, Everett H. Pryde, Ordean L. Brekke, Timothy L. Mounts, and Richard A. Falb. American Soybean Association and American Oil Chemists' Society Publishers, Champaign, Illinois, 1980, Ch. 20, pp. 439-458.

EMKEN, E. A. Nutritional Aspects of Soybean Oil Utilization. In: World Soybean Research Conference II: Proceedings, edited by Frederick T. Corbin, pp. 667-679, Westview Press, Boulder, Colorado, 1980.

EMKEN, E. A. Influence of *trans*-9-, *trans*-12-, and *cis*-12-Octadecenoic Acid Isomers on Fatty Acid Composition of Human Plasma Lipids. Progress in Lipid Research, accepted November 1980.

EMKEN, E. A. Metabolic Aspects of Monounsaturated Fatty Acid Isomers. J. Am. Oil Chem. Soc., accepted November 1980.

LANSER, A. C. AND E. A. EMKEN. Competitive Deposition of trans-12- and cis-9-Octadecenoates Into Egg Yolk Lipids. Lipids. Accepted October 1980.

SELKE, E., W. K. ROHWEDDER, and H. J. DUTTON. Volatile Components from Trilinolein Heated in Air. J. Am. Oil Chem. Soc. 57 (1980):25-30.

Other Reports:

DeJARLAIS, W. J. Comparison of Acetylenic and Wittig Syntheses of Hexadenterio-11-Octadecenoates. Presented at the American Chemical Society 179th National Meeting, Houston, Texas, March 23-28, 1980.

DeJARLAIS, W. J. Synthesis of Deuterated Isomers. Presented at Review of Soybean Research, Northern Regional Research Center, Peoria, Illinois, May 15-16, 1980.

DUTTON, H. J. Incorporation of Isomeric Fatty Acids in Egg and Human Blood Lipids. Presented at the combined meeting of the International Society for Fat Research/American Oil Chemists' Society, New York, New York, April 27-May 1, 1980.

EMKEN, E. A. Metabolic Aspects of Monounsaturated Fatty Acid Isomers. Presented at the World Conference on Soya Processing and Utilization, Acapulco, Mexico, November 9-14, 1980.

EMKEN, E. A. Influence of trans-9, trans-12-, and cis-12-Octadecenoic Acid Isomers on Fatty Acid Composition of Human Plasma Lipids. Presented at Golden Jubilee International Congress on Essential Fatty Acids and Prostaglandins, University of Minnesota, Minneapolis, Minnesota, May 5-7, 1980.

EMKEN, E. A. Fate of Isomeric Octadecenoic Acids in Human Blood Lipids. Presented to the meeting of the Technical Committee of the Institute of Shortening and Edible Oils, Peoria, Illinois, September 17, 1980.

EMKEN, E. A. Use of Deuterium Labeled Fatty Acids in Human Studies. Presented in Seminar to the Lipid Nutrition Laboratory, Beltsville, Md., November 24, 1980.

EMKEN, E. A. Biochemical Behavior of trans Acids. Presented at Program Review of Soybean Research, Northern Regional Research Center, Peoria, Illinois, May 15-16, 1980.

LANSER, A. C. Comparative Deposition of trans-Octadecenoates in Egg Lipids. Presentation to the Technical Committee of the Institute of Shortening and Edible Oils, Peoria, Illinois, September 17, 1980.

OHLROGGE, J. B. Occurrence of Octadecenoate Positional and Geometric Isomers in Humans. Presented at Soybean Program Review, May 16, 1980, Peoria, IL.

OHLROGGE, J. B. Human Lipid Composition: Occurrence of Fatty Acid Isomers from Dietary Hydrogenated Oils. Presented at Shortening Institute Technical Committee Meeting, September 17, 1980, Peoria, IL.

OHLROGGE, J. B. Hydrogenated Vegetable Oils: Are They Incorporated into Human Tissue? Presented at Joint Meeting of American Chemical Society and Sigma Xi, December 11, 1980, Peoria, IL.

RAKOFF, H. AND E. A. EMKEN. Stereoselective Control of the Wittig Reaction: Preparation of Methyl cis,cis-, cis,trans-, trans,cis, and trans,trans-12,15-Octadecadienoate-9,10-d₂. Presented at ISF/AOCS World Congress, New York City, New York, April 27-May 1, 1980.

RAKOFF, H., D. WEISLEDER, AND E. A. EMKEN. ¹³C Nuclear Magnetic Resonance Spectroscopy of the Four Methyl 12,15-Octadecadienoate Geometric Isomers. Presented at ISF/AOCS World Congress, New York City, New York, April 27-May 1, 1980.

ROHWEDDER, W. K. Chemical Ionization Selected-Ion-Monitoring Mass Spectrometric Analysis of Deuterium Triple-Labeled Blood Lipids. Presented at the American Society for Mass Spectrometry, New York, NY, May 25-30, 1980.

ROHWEDDER, W. K. Analytical Methods. Presented at Soybean Program Review, Peoria, IL, May 16, 1980.

NORTHERN AGRICULTURAL ENERGY CENTER

M. O. Bagby, Acting Manager

A. TECHNOLOGIES FOR INDUSTRIAL USES FOR
PLANT AND ANIMAL PRODUCTS

1. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. O. Bagby)

- a. Specific Objective: Identify and call attention to plant species that are potentially adaptable to U.S. agricultural practice and have an overall composition favoring their economic development as multi-use botanochemical-producing crops.

Progress: The NRRC botanist collected 164 whole-plant samples mainly from Illinois. Cooperating USDA plant scientists provided four species of the Euphorbiaceae family from Florida and 12 range species from Arizona. Species from 10 previously unevaluated families were screened although major emphasis was with about 90 species of the Leguminosae family. Rhus laurina, Asclepias hirtella, Xylococcus bicolor, Euphorbia lathyris, and Sapium candatum contained 5 to 9% whole-plant oil and Asclepias tuberosa had greater than 2% rubber.

- b. Specific Objective: Characterize oils, polyphenols, and hydrocarbon polymers produced by select U.S. plant species.

Progress: Typical of previously examined whole-plant oil extracts, saponification of extractives from Asclepias hirtella liberated 47% free acid and from Xylococcus bicolor 32%. The esters were mainly nonglyceride. Rubbers extracted from A. hirtella and X. bicolor have molecular weights of about 10 and 30%, respectively, that of Hevea.

- c. Specific Objective: Cooperate with university and USDA scientists in plant breeding efforts to develop botanochemical crop varieties.

Progress: Common milkweed (Asclepias syriaca) plant samples (50) were collected in Maryland by a USDA agronomist and analyzed for extractives variability. Total extractives ranged from 5 to 24% while less variability was observed for the hydrocarbon portion. Similarly, smooth sumac (Rhus glabra) plant samples (100) were analyzed. Total acetone solubles ranged from 20 to 45% and the hydrocarbon content ranged from nearly zero to 6%.

- d. Specific Objective: Evaluate cellulosic and lignocellulosic residues from species identified as promising renewable sources of valuable phytochemicals.

Progress: Sunflower (*Helianthus annuus*) crop residue was collected in Minnesota. The majority of its fibers were 0.5 to 1.8 mm long. The wet-cleaned residue contained 16% lignin, 25% pentosan, 59% cellulose, and 39% alpha-cellulose.

- e. Specific Objective: Evaluate various plant resins, oils, and hydrocarbons for potential industrial utility.

Progress: Seventeen species identified to have promising quantities of high-energy extractables have been recollected and submitted to a university cooperator to determine heats of combustion and gasification parameters.

- f. Specific Objective: Evaluate fiber residues as phytochemical materials.

Progress: Fibrous residues from the commercially important sunflower (*Helianthus annuus*), guayule (*Parthenium argentatum*), candelilla (*Euphorbia antisiphilitica*), and creosote bush (*Larrea tridentata*) were evaluated as papermaking fiber resources. Sunflower provided 43 to 48% of readily bleachable soda pulp with strength properties equal to or exceeding those of typical sugarcane bagasse pulp. The guayule, candelilla, and creosote bush provided poor quality pulps in about 30% yield.

Publications:

BAGBY, M. O., R. A. BUCHANAN, AND F. H. OTEY. Multi-Use Crops and Botanochemical Production, invited ACS Monograph Chapter, in press.

BUCHANAN, R. A., F. H. OTEY, AND G. E. HAMERSTRAND. Multi-Use Botanochemical Crops, An Economic Analysis and Feasibility Study, invited chapter, in press.

TOUZINSKY, G. F., R. L. CUNNINGHAM, AND M. O. BAGBY. Papermaking Properties of Kenaf Thermomechanical Pulp. *Tappi* 63(1) (1980): 53-55.

TOUZINSKY, G. F., R. L. CUNNINGHAM, AND M. O. BAGBY. Laboratory Paper Machine Runs with Kenaf Thermomechanical Pulp. *Tappi* 63(3) (1980): 109-110.

Other Reports:

BAGBY, M. O. Hydrocarbon Crops. Invited presentation, Scientific Committee Corn Refiners Association, Inc., NRRC, Peoria, Illinois, November 13, 1979.

BAGBY, M. O. Northern Agricultural Energy Center, Crop Production Research for Alcohol Fuel Workshop, Atlanta, Georgia, April 8-9, 1980.

BAGBY, M. O. Hydrocarbon Crops. Invited presentation, Twenty-First Annual Corn Dry Milling Conference, NRRC, Peoria, Illinois, June 3-4, 1980.

BAGBY, M. O. Phytochemicals: Nature's Materials Resource. Invited presentation, 11th Akron Polymer Conference on Polymers for Improved Energy Efficiency, Akron, Ohio, June 5-6, 1980.

BAGBY, M. O. Hydrocarbon Crops. Invited presentation, NCR-114 Committee Crop Residues as an Alternate Energy Source, Peoria, Illinois, June 30, 1980.

BAGBY, M. O. SEA Regional Energy Centers--Organization and Programs. Invited presentation, AES Collaborators Conference, NRRC, Peoria, Illinois, October 20-21, 1980.

BAGBY, M. O. Herbs and Grasses for Fuel and Chemicals. Invited presentation, Bloomington-Normal Club of Sigma Xi, Illinois State University, Normal, Illinois, November 4, 1980.

ROTH, W. B., R. A. BUCHANAN, I. M. CULL, L. L. SWANSON, AND M. O. BAGBY. Hydrocarbon and Rubber-Producing Crops: Evaluation of a Third Set of 100 Species, Economic Botany Society, Bloomington, Indiana, June 15-18, 1980.

Numerous interviews for trade journals, television, radio, and newspaper releases.

2. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. W. Detroyn)

- a. Specific Objective: Isolate a mutant of Bacillus species which is capable of starch hydrolysis when cultivated in the presence of glucose and is not subject to glucose repression.

Progress: Temperature-sensitive mutants have been and are being evaluated for starch hydrolysis of elevated temperatures.

- b. Specific Objective: Study chemical and physical treatments of starch to lower energy requirements for starch gelatinization.

Progress: Several corn starch derivatives of low DS (e.g., <0.01 to 0.05) were prepared and interacted with milled barley malt. The derivatives offered no advantages over the original starch. In subsequent work, native corn starch was lignified and saccharified under a variety of conditions using combination of bacterial alpha-

amylase and glucoamylose (Taka-Therm and Diazyme, commercial preparations). Preliminary results proved encouraging, and this promising lead is being further evaluated as a continuous process.

- c. Specific Objective: Increase efficiency of saccharification of lignocellulosic residues to fermentable sugars.

Progress: Wheat straw has been modified by chemical, physical, and thermal techniques to improve the conversion of cellulose to fermentable sugars by enzymatic hydrolysis. Purification of the cellulosic component by chemical or chemical/thermal processes has improved the efficiency of saccharification. Treatments included various concentrations of H_2SO_4 and NaOH followed by secondary treatments with ethylene diamine (EDA) and NH_4OH prior to enzymatic saccharification. Conversion of the cellulosic component to sugar varied with the chemical modification steps. Treatment solely with alkali yielded 51 to 75% conversions, depending upon temperature. Acid treatment at elevated temperatures showed a substantial decrease in the hemicellulose component, whereas EDA-treated WS (acid-pretreated) showed a 69-75% decrease in the lignin component. Acid-pretreated EDA-treated straw yielded a 98% conversion rate, followed by 83% for alkali- NH_4OH treated straws.

In other experiments, WS was pretreated with varying concentrations of H_2SO_4 or NaOH followed by NH_4OH treatment prior to enzymatic hydrolysis. Pretreatment of straw with 2% NaOH for 4 hr coupled to enzymatic hydrolysis yielded a 76% conversion of the cellulosic component. Acid-base combination pretreatments yielded only 43% conversions.

A reactor column was subsequently employed to measure modification-saccharification fermentation for wheat straw conversion on a larger scale. Thirty percent conversions of wheat straw cellulosics to sugar were observed with subsequent fermentation to alcohol. The crude cellulase preparation yielded considerable quantities of xylose in addition to the glucose. Saccharified materials were fermented directly with actively proliferating yeast cells without concentration of the sugars.

- d. Specific Objective: Evaluate fungi for ability to preferentially degrade lignin in lignocellulosics.

Progress: Differentially ^{14}C -labeled, naturally complexed lignocelluloses (LC) were employed to evaluate selected fungi for their ability to hydrolyze cellulose and degrade and solubilize lignin subsequent to alcohol fermentation. Eight fungal isolates from collections of cattle dung representing different stages in decomposition were examined for their ability to biologically modify the lignin and cellulose components of unamended native wheat straw (WS). Growth rate of fungal isolates on dung extract

agar was not correlated with their ability to affect biomass loss of WS. Later successional (sporulation) colonists promoted greater biomass losses (total dry weight and cellulose). A late-appearing unidentified basidiomycete was most effective in degrading lignin and rendering the cellulosic components of natural lignocelluloses more susceptible to hydrolysis by cellulase. Fungal degradation of differentially ^{14}C -labeled natural lignocelluloses (LC) added to ground and pelletized WS was followed by monitoring $^{14}\text{CO}_2$ evolution during a 48-day incubation. Three late-appearing species on dung were from 20-47% more efficient in producing $^{14}\text{CO}_2$ from specifically cellulose labeled (LC*) than were the four earlier appearing successional species tested. The percentage of $^{14}\text{CO}_2$ evolved from lignin labeled (L*C) was sixfold greater with the late-appearing basidiomycete than with any of the six ascomycetes tested. Present findings help to explain patterns of fungal development in cattle feces and the importance of lignin-degrading, "white rotting" basidiomycetes in feces decomposition.

- e. Specific Objective: Apply molecular genetics technology to the improvement of alcohol production by bacteria and/or yeasts from plant polysaccharides.

Progress: A bio-hazards recombinant DNA laboratory facility has been constructed. Experimentation is beginning in cell fusion technology and recombinant DNA.

Anticipated research centers around producing recombinant organisms more efficient in the production of plant polysaccharide-degrading enzymes, fermentation enzymes, cellulase, cellobiase, amylase, and other enzymes, utilizing new technology in cell fusion and recombinant DNA.

- f. Specific Objective: Initiate studies on the role(s) of laccase and peroxidase in the biodegradation of lignin. Examine the organisms for the production of a beta-etherase. Initiate selection and mutation studies of these organisms for improved nutrient and feedstuff production.

Progress: Selected mesophilic and thermophilic fungi were investigated for their ability to degrade lignin and/or convert the cellulose component of wheat straw into a saccharifiable state. The degradation studies were performed with specifically labeled ^{14}C -lignocellulose. The organisms were grown on the substrate and the $^{14}\text{CO}_2$ released was measured. The release of cellulose for saccharification by the cellulase enzyme complex was measured by cellulase treating a given dry weight of fermented wheat straw and measuring the released reducing sugars. From these studies, the organism that appeared most promising was Cyathus stercoreus.

After uniform reproducible inoculation procedures were devised, studies were initiated to optimize the fermentation conditions. Various carbon and nitrogen sources, vitamins, reported lignolytic enzyme inducers, and moisture and temperature conditions were tested. The amount of saccharifiable cellulose present at various times throughout the fermentation was measured. These studies revealed that the optimal fermentation conditions are 80% moisture and 28°C. None of the additions tested increased the yield of saccharifiable cellulose.

In order to reduce the time required for the fermentation of wheat straw, attempts to adapt the fungus to this substrate were made by continual propagation of the organism on this substrate. The organism did adapt such that, by 9 days, the organism had reduced the lignin component by 40-50% and the cellulose component by 15-20%. Of the 80% remaining cellulose, 70% was saccharifiable. Before adaptation, approximately 30 days was required to obtain comparable results.

Preliminary experiments on the role of laccase, peroxidase, and tyrosinase in lignin degradation indicate that laccase and peroxidase are unimportant. These enzymes were not detected in the fermenting of wheat straw; however, easily detectable levels of tyrosinase were found.

- g. Specific Objective: Mutate Phanerochaete chrysosporium for loss of cellulase activity without loss of ability to degrade lignin.

Progress: Cellulase (-) presumptive isolates have been obtained by UV treatment and are being subjected to further analysis.

- h. Specific Objective: To investigate fungi and bacteria that produce amylases sufficient to break down raw corn starch to glucose.

Progress: The following microorganisms were found to produce glucose from cracked corn: Bacillus marcerans NRRL B-430, B. subtilis NRRL B-3696, B. subtilis NRRL B-645; Aspergillus oryzae NRRL 468, A. awramori NRRL 3112, A. foetidus NRRL 337. The two most promising microorganisms were the bacteria B. marcerans and the fungus Aspergillus foetidus. Presoaked cracked corn inoculated with A. foetidus routinely converted 20% starch to glucose in 3 days while B. marcerans converted about 11% of the available starch to glucose. The other microorganisms converted about 2% of the starch to glucose in the same time period (3 days). Presoaking the cracked corn in Ca + KH₂PO₄ increased by 2% the glucose produced. Amylase activity apparently peaks at 3 days and drops to zero at day 7 and then again exponentially rises to a maximum at day 14.

Publications:

CUNNINGHAM, R. L., M. O. BAGBY, AND R. W. JUGENHEIMER. Sweet-Stalked Corn. *Transactions of the Illinois State Academy of Science*, in press.

DETROY, R. W., L. A. LINDENFELSER, G. ST. JULIAN, JR., AND W. L. ORTON. Saccharification of Wheat Straw Cellulose by Enzymatic Hydrolysis Following Fermentative and Chemical Pretreatment. *Biotechnol. Bioeng. Symp.* No. 10 (1980):135-148.

DETROY, R. W. AND C. W. HESSELTINE. Fermentation Products of Plant Materials. In *Handbook of Agriculture, Processing and Utilization*, ed. Dr. I. Wolff, CRC Press, 1980, in press.

DETROY, R. W. AND R. H. RHODES. Biological Conversion of Agricultural Lignocellulosics to Ethanol and Digestible Feedstuffs. *EOCD Proc. Energy Symp.*, Amersfoort, Netherlands.

WICKLOW, D. T., R. W. DETROY, AND B. A. JESSEE. Decomposition of Lignocellulose by Cyathus stercoreus (Schw.) de Toni NRRL 6473, a "White Rot" Fungus from Cattle Dung. *Appl. Environ. Microbiol.* 40(1980) 169-170.

Other Reports:

CUNNINGHAM, R. L. Kenaf Biomass Harvest from Experimental Plots on Stripmine Land. Invited presentation, Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, Illinois, February 7, 1980.

CUNNINGHAM, R. L. AND M. O. BAGBY. Sweet-Stalked Corn. Abstract of Papers, 73rd Annual Meeting, Illinois State Academy of Science, Lisle, Illinois, April 18-19, 1980, 3.

CUNNINGHAM, R. L. Biomass and Energy Research at the Northern Agricultural Energy Center. Invited presentation, Biomass Coordinating Meeting, Metropolitan Sanitary District of Greater Chicago, Wee-Ma-Tuk Country Club, Fulton County, Illinois, July 1, 1980.

CUNNINGHAM, R. L. Progress Report on the 1980 Kenaf Crop Grown on Stripmine Land Amended with Sewage Sludge. Invited presentation, Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, Illinois, November 14, 1980.

DETROY, R. W. AND S. N. FREER. Biological Delignification of ¹⁴C-Lignocelluloses by Basidiomycetous Fungi. Presented at the ASM meeting, Miami Beach, Florida, May 11-17, 1980.

DETROY, R. W. Biological Conversion of Agricultural Lignocellulosics to Ethanol and Digestible Feedstuffs. Presented at the EOCD Energy Symposia, Amersfoort, Netherlands, October 6-10, 1980.

DETROY, R. W. Fermentation of Plant Polysaccharides: Role of Biochemical Genetics. Presented at Symposium on Trends in the Biology of Fermentations for Fuels/Chemicals at Brookhaven National Laboratory, Upton, New York, December 7-11, 1980.

DETROY, R. W., G. ST. JULIAN, AND S. N. FREER. Chemical and Biological Conversion of Agricultural Residues for Energy and Feedstuffs. Presented at the SW/SE Regional ACS meeting, New Orleans, Louisiana, December 9-13, 1980.

3. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

- a. Specific Objective: Develop an efficient process that converts aflatoxin-contaminated corn to alcohol and renders the spent grain safe for animal feed.

Progress: Zymomonas mobilis demonstrated greater fermentative activity than Saccharomyces uvarum during the first day in the fermentation of two lots of aflatoxin-contaminated corn and two corresponding lots of ammonia-detoxified corn. Final ethanol yields and conversion efficiencies were generally highest in Zymomonas fermentations of ammonia-detoxified corn. Aflatoxin levels in post-fermentation solids from ammonia-detoxified corn all ranged below the FDA feedstuff guideline of 20 ppb, whereas the amount of aflatoxin in post-fermentation solids from aflatoxin-contaminated corn was greater than in the initial corn.

When ammonia was applied at a rate of 0.5-2.0% db, as ammonium hydroxide during the liquefaction of aflatoxin-contaminated corn, 85% of the aflatoxin was destroyed and better ethanol yields were obtained than when no ammonia was added during liquefaction.

- b. Specific Objective: Evaluate selected microorganisms and conditions for increased alcohol and chemical feedstock yields from xylose.

Progress: In lignocellulosic materials there is a hemicellulose fraction yielding five carbon sugars that are not readily fermentable. A systematic search of yeasts from the AR Culture Collection has uncovered a yeast that has the unique capability to efficiently convert D-xylose to ethanol. An invention report has been filed on a process for producing ethanol by fermentation of pentose sugars by yeasts. Applications of the process are being developed.

- c. Specific Objective: Determine immobilized cell process for conversion of plant polymer sugar to alcohol.

Progress: Two yeasts, Saccharomyces cerevisiae NRRL Y-2034 and Saccharomyces uvarum NRRL Y-1347, and the bacterium Zymomonas mobilis NRRL B-806 were bound in Na-alginate gel to prevent the microorganisms from wash-out in continuous flow fermentation experiments. A continuous-flow fermentor was designed so that a glucose solution (10%) could flow slowly (3 ml/hr) by the immobilized microorganisms and then into a collecting vessel at a dilution rate of 0.026 h⁻¹. This immobilization technique enabled the microorganisms to continuously ferment glucose to alcohol for several days. Problems of uneven distribution of the immobilized cell beads and channeling of the substrate were eliminated by the development of a multistage apparatus. Results showed that ethanol production in continuous fermentation using a multistage apparatus continued at 100% conversion of glucose to ethanol after 12 days for S. cerevisiae, and 80% after 12 days for S. uvarum.

- d. Specific Objective: Select and test various microorganisms from the AR Culture Collection for use in continuous culture experiments aimed at improving alcohol production.

Progress: One strain showing promise for future use in continuous fermentations is the nonflocculating yeast Saccharomyces cerevisiae NRRL Y-2235. Ethanol was produced rapidly by this organism in batch fermentations having initial glucose concentrations ranging from 5 to 30%. When grown in batch culture on a medium containing 20% glucose, NRRL Y-2235 was able to consume all of the sugar present in less than 48 hours and to yield ethanol in a final concentration of 9.4% (by weight). Assuming that 1 mole of glucose forms 2 moles of ethanol, the alcohol yield in this instance was 94% of the theoretical. Even when the initial glucose concentration was 30%, NRRL Y-2235 consumed all of the sugar available and produced an alcohol yield to 88% of the expected theoretical yield.

- e. Specific Objective: Test several amylolytic microorganisms for the ability to hydrolyze raw or modified starch.

Progress: In fermentation, a major energy input is in the conversion of raw starch to sugars. A number of microorganisms, including bacteria and fungi, that act effectively on raw starch have been found and are being investigated in detail. Through the coupling of fungi (for conversion of the starch to sugar) with other microorganisms (for fermentation), there are excellent opportunities to develop low-energy in-put solid substrate fermentation processes, which have been neglected in the past when the need was for potable alcohol.

- f. Specific Objective: Develop low-energy alternative to costly high-temperature drying for the preservation of wet distillers feed grains.

Progress: Distillers feed grains are valuable fermentation byproducts but are recovered wet and are extremely perishable. A number of preservatives which would avoid the need to dry these residues have been tested. For example, sorbic acid at treatment levels of 0.5 and 1.0% (wt/wt) effectively preserved wet (35% solids) distillers grains through 21 days of storage at 28°C.

Under these same conditions, stillage treated with 0.25% (wt/wt) sorbic acid was slightly deteriorated by 8 days, while untreated stillage was extremely moldy by 8 days. Similar data are being collected for propionic acid, potassium sorbate, ammonia, sulfur dioxide, carbon dioxide, etc.

Publications:

NOFSINGER, G. W. AND R. J. BOTHAST. Ethanol Production by Zymomonas Mobilis and Saccharomyces uvarum on Aflatoxin-Contaminated and Ammonia-Detoxified Corn. Can. J. Microbiol. In press.

Other Reports:

BOTHAST, R. J. Ethanol Fermentation Research. Invited presentation, NCR-114 Committee Crop Residues as an Alternate Energy Source, Peoria, Illinois, June 30, 1980.

BOTHAST, R. J. Innovative Fermentation Technology for Alcohol Production. Presented at the Peoria Section, American Chemical Society, Peoria, Illinois, January 22, 1980.

BOTHAST, R. J. Northern Agricultural Energy Center on Alcohol and Production of Biomass Feedstocks. Presented at Bio-Energy '80, Atlanta, Georgia, April 22, 1980.

BOTHAST, R. J. Northern Agricultural Energy Center Program. Presented at a Program Workshop on Fuels and Energy from Biomass: A Critical Reveiw of the Progress Being Made in Illinois, Urbana, Illinois, August 25, 1980.

BOTHAST, R. J. Ethanol Production from Grain and Sugar Crops. Presented at a Symposium on Fuel from Crops at the 72nd Annual Meeting of the American Society of Agronomy, Crop Science Society of America, and Soil Science of America, Detroit, Michigan, December 1, 1980.

MCGHEE, J. E. Energy from Renewable Sources. Presented to Eastern Illinois Department of Botany, Charleston, Illinois, March 19, 1980.

MCGHEE, J. E. Energy from Renewable Sources: Immobilized Cell Technology in Ethanol Fermentation. Presented at Ball State

University, College of Sciences and Humanities, Muncie, Indiana,
October 21, 1980.

4. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

- a. Specific Objective: To determine composition of distillation residues (stillage) and its fractions from ethanol fermentation of grain.

Progress: The stillage from corn alcohol fermentation was separated into supernatant and residue by screening and centrifugation.

The supernatant contained primarily degraded materials smaller than 10,000 in molecular weight and accounted for 20% of the dry weight and 20% of the total nitrogen. Free amino acids accounted for 5.6% of the nitrogen in the total residual material and 28% of the nitrogen in the supernatant. The protein in the residue was considerably less soluble than that of ground corn when a series of solvents was used for fractionation, probably due to heat denaturation. The lowered solubility of the protein in the residue may reduce degradation of protein in the rumen and favor digestion and absorption of a higher proportion from the lower gastrointestinal tract so that growth and feed efficiency are increased for ruminants.

- b. Specific Objective: Devise methods for fractionating, drying and utilizing valuable feed materials from methane fermentation media.

Progress: Typical methane fermenter effluent (MARC, Clay Center) contains 8% total solids (TS) which is 28% protein ($N \times 6.25$) and 13% ash (dry basis). Wet sieving on a 60 mesh screen isolates 30% of the fermenter effluent TS which contains 10% protein and 3% ash (dry basis). Centrifuging the -60 mesh fraction at 1100 X g removes 22% of the TS in original system, and centrifuging the centrate at 12,000 X g removes a further 6% TS. The combined centrifuge cakes contain 24% protein and 8% ash (dry basis). The final centrate is a slightly turbid solution containing 2.5% TS which is 7% N and 23% ash dry basis. The centrate contains 26% of the TS, 39% of the N, and 44% of the ash present in the original effluent. Half of the centrate N is NH_3 or volatile amines. Trichloroacetic acid only removed 7% of the TS. The nitrogen in the centrate is contained in low molecular weight compounds rather than protein or microbial cells.

- c. Specific Objective: Develop procedures to recover and upgrade protein from distillers feed grains and solubles and to permit recycling of water.

Progress: Preliminary results indicate that pin-milling of corn distillers dried grains with solubles and then sieving through

selected screens yields fractions that differ greatly in protein content. Allowing stillage from fermentation of corn to settle under gravity resulted in layering of the solids to give an upper 42% protein fraction and a lower 18% protein fraction. The lower fraction had a higher proportion neutral detergent fiber (57% vs. 21%). Preliminary studies employing a liquid cyclone apparatus gave encouraging results that indicate that apparatus could be economically used to produce high-protein fractions with reduced fiber from stillage on a commercial scale. The distillers solubles (centrifuged liquid fraction of stillage) was recycled into the mashing and fermentation liquids of subsequent corn fermentations. After several fermentations using the recycled solubles, no decline in alcohol yield was observed. However, a buildup in solids content of the stillage took place. The reuse of stillage reduced energy costs in drying and yielded a more concentrated solubles solution that was more economical to recover.

Publications:

WU, Y. V., K. R. SEXSON, AND J. S. WALL. Protein-Rich Residue from Corn Alcohol Distillation: Fractionation and Characterization. Cereal Chemistry. Submitted for publication.

Other Reports:

WU, Y., K. R. SEXSON, AND J. S. WALL. Protein-Rich Residue from Grain Alcohol Distillation: Fractionation and Characterization. Presented at the 65th Annual Meeting, American Association of Cereal Chemists, Chicago, Illinois, September 21-25, 1980.

BAGLEY, E. B. Agricultural Energy Center. Invited presentation, Twenty-First Annual Corn Dry Milling Conference, NRRC, Peoria, Illinois, June 3-4, 1980.

BAGLEY, E. B. Northern Agricultural Energy Center. Invited presentation, NCR-114 Committee Crop Residues as an Alternate Energy Source, Peoria, Illinois, June 30, 1980.

UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION
AGRICULTURAL RESEARCH
NORTHERN REGIONAL RESEARCH CENTER
1815 NORTH UNIVERSITY STREET
PEORIA, ILLINOIS 61604

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF AGRICULTURE
AGR-101



OFFICIAL BUSINESS

